Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC
Draw Desc | Image

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☐ 4. Document ID: US 6080766 A

L4: Entry 4 of 11 File: USPT Jun 27, 2000

US-PAT-NO: 6080766
DOCUMENT-IDENTIFIER: US 6080766 A

TITLE: Method for the treatment of fibroproliferative disorders by application of inhibitors of protein hydroxylation

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC
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☐ 5. Document ID: US 6046219 A

L4: Entry 5 of 11 File: USPT Apr 4, 2000

US-PAT-NO: 6046219
DOCUMENT-IDENTIFIER: US 6046219 A

TITLE: Method for the treatment of conditions mediated by collagen formation together with cell proliferation by application of inhibitors of protein hydroxylation

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC
Draw Desc | Image

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☐ 6. Document ID: US 6020139 A

L4: Entry 6 of 11 File: USPT Feb 1, 2000

US-PAT-NO: 6020139
DOCUMENT-IDENTIFIER: US 6020139 A

TITLE: S-adenosyl methionine regulation of metabolic pathways and its use in diagnosis and therapy

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 7. Document ID: US 5965586 A

L4: Entry 7 of 11 File: USPT Oct 12, 1999

US-PAT-NO: 5965586
DOCUMENT-IDENTIFIER: US 5965586 A

TITLE: Method for the treatment of fibroproliferative disorders by application of inhibitors of protein hydroxylation

L4: Entry 2 of 11 File: USPT Apr 17, 2001

DOCUMENT-IDENTIFIER: US 6217864 B1
TITLE: Method for targeted degradation of intracellular proteins in vivo or ex vivo

Detailed Description Text (167):
For this study, cells were transformed as above and treated with the polyamine precursor putrescine for the indicated times to induce antizyme. ODC activity in the form of M314T-p53 100-150 was regulated by polyamines. Results are seen in FIG. 11.

Detailed Description Text (169):
To elicit polyamine-mediated regulation and induce endogenous antizyme, putrescine was added to the culture medium of cells expressing M314Tb or M314Tb-p53 100-150 to a final concentration of 100 .mu.M. Cell lysates were prepared after the indicated time of treatment and assayed for ODC activity as described in Mol.Cell Biol., 13:2377-2388 (1993). Control cells were similarly incubated, harvested, and analyzed but were not treated with putrescine. ODC activities are represented as the percentage of initial activity. M314Tb and M314Tb-p53 100-150 initial activities were 72.9 and 6.6 pmol/mg/min, respectively. M314T is depicted as circles. M314T-p53 100-150 is depicted as triangles. Treated cells are depicted as solid symbols. Untreated control cells are depicted as open symbols.

Detailed Description Text (196):
Polyamines are small organic polycations ubiquitous in living organisms. They are essential for cellular proliferation and development. The biochemical functions of polyamines are diverse. Optimal concentrations of polyamines benefit the fidelity and efficiency of in vitro transcription and translation reactions. This suggests that the rapid changes in polyamine pools observed in response to cellular signals for growth or differentiation are needed to optimize these biosynthetic processes. They are present in millimolar concentrations and are largely bound to RNA and DNA. Spermidine participates in a unique postranslational modification of the protein eIF-5A. Preventing that modification by genetic or pharmacological manipulation is lethal to cells.

Detailed Description Text (199):
The subject of the treatment is pretreated, for example, with polyamine compounds such as spermine, spermidine, putrescine, cadaverine or their analogs in doses which increase antizyme production. Alternatively, the antizyme is produced extracorporally and delivered into the cells or tissue.

Detailed Description Text (325):
Constructs TbODC-p53 100-150 and M314T-p53 100-150 were cloned into a mammalian expression vector under the control of the SV40 early promoter according to Genes and Dev.,4:764-778 (1990). The plasmids, along with pMC1 conferring neomycin/G418 resistance were prepared according to Cell,51:503-512 (1987), were used to transfect mutant Chinese hamster ovary C55.7 cells devoid of endogenous ODC activity according to J. Biol. Chem., 257:4603-4509 (1982). Transformants were obtained by subjecting the cells simultaneously to two forms of selection: 1) with the drug G418, selective for cells expressing the neo gene encoded by pMC1, and 2) by incubation in polyamine-deficient medium, selective in the mutant ODC-deficient cells for expression of the transfected ODC gene, which is needed for polyamine biosynthesis. Pools of stably transformed clones were plated (approximately 10.sup.6 cells/100-mm Falcon dish) and incubated at 37.degree. C. for 16 hours. To elicit polyamine-mediated regulation and induce endogenous antizyme, putrescine was added

to the culture medium to a final concentration of 100 um. ODC stability was assessed in some experiments by inhibiting protein synthesis with cycloheximide, 100 mg/ml of cell culture medium, and determining the rate of decay of enzymatic activity. Cell lysates were prepared after the indicated time of treatment and assayed for ODC activity as described in Mol.Cell Biol, 13:2377-2388 (1993).

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

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☐ 8. Document ID: US 5965585 A

L4: Entry 8 of 11 File: USPT Oct 12, 1999

US-PAT-NO: 5965585
DOCUMENT-IDENTIFIER: US 5965585 A

TITLE: Method for the treatment of fibroproliferative disorders by application of inhibitors of protein hydroxylation

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

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☐ 9. Document ID: US 5849587 A

L4: Entry 9 of 11 File: USPT Dec 15, 1998

US-PAT-NO: 5849587
DOCUMENT-IDENTIFIER: US 5849587 A

TITLE: Method of inhibiting viral replication in eukaryotic cells and of inducing apoptosis of virally-infected cells

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

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☐ 10. Document ID: US 5789426 A

L4: Entry 10 of 11 File: USPT Aug 4, 1998

US-PAT-NO: 5789426
DOCUMENT-IDENTIFIER: US 5789426 A

TITLE: Method for the treatment of fibroproliferative disorders by application of inhibitors of protein hydroxylation

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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| Term | Documents |
|---|---|
| EIF-5A.DWPI,EPAB,JPAB,USPT,PGPB. | 36 |
| EIF-5AS | 0 |
| (EIF-5A AND 3).USPT,PGPB,JPAB,EPAB,DWPI. | 11 |
| (L3 AND EIF-5A).USPT,PGPB,JPAB,EPAB,DWPI. | 11 |

**Display Format:** - Change Format

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L4: Entry 3 of 11 File: USPT Jan 9, 2001

DOCUMENT-IDENTIFIER: US 6172261 B1
TITLE: Polyamine analogues as therapeutic and diagnostic agents

Brief Summary Text (11):
Augmented PAT into cancer cells promotes cell killing. J. L. Holley et al. (Cancer Res. 52:4190-4195, 1992) showed up to a 225-fold increase in cytotoxicity of a chlorambucil-spermidine conjugate compared to chlorambucil alone. A series of nitroimidazole-polyamine conjugates were also effective (Holley, J. L. et al., Biochem. Pharmacol. 43:763-769, 1992). Others showed that mice infected with a multi-drug resistant strain of malaria were cured by treatment with a chloroquinoline-putrescine conjugate (Singh, S. et al., J. Biol. Chem. 272:13506-13511, 1997). Thus, the effectiveness of cytotoxic compounds could be enhanced by their conjugation with polyamines. These effects may have been due to the exploitation of the PAT system to deliver these compounds into cancer cells. The present invention is therefore directed in part to rapid and efficient testing of many different conjugates between polyamines and known drugs for their transport into cells. Furthermore, as described below, this invention combines the cytotoxic properties of known drugs with the facilitated transport of polyamines, which relies on the present inventors' discoveries surrounding the PATr described herein. By accessing the database of structure-activity-relationships (SARs) of PATr substrates, the present inventors are able to predict the transportability of a novel chemical entity or a novel polyamine conjugate.

Brief Summary Text (14):
The radiometric assay uses radiolabeled polyamines such as putrescine, spermidine or spermine, but, due to the low signal, large numbers of adherent or non-adherent cells are required. Additional care is required with spermine due to its non-specific adsorption to cells and plastics. Cells are mixed with the test compounds and the radiolabeled polyamine to initiate the assay. The cells are incubated for 1-60 minutes, depending on cell type. The assay is terminated by removal of the medium and cooling the plates to 4.degree. C. The cells are then washed with cold medium three times, dissolved in 0.1% sodium dodecyl sulfate and the radioactivity in solution is then determined by scintillation counting. This assay is difficult to scale up to a high throughput procedure due to the low signal from the radiolabel and the handling requirements inherent in procedures with radioactivity.

Brief Summary Text (23):
The protein eIF-5A appears to play a role in protein synthesis, although its exact function remains obscure (Hanauske-Abelm, H. M. et al., FEBS Lett. 266:92-98, 1995). EIF-5A is unique in that it is modified by the unusual amino acid hypusine. Hypusine is generated post-translationally by the sequential action of deoxyhypusyl synthase (using spermidine as a substrate) and deoxyhypusyl hydroxylase. Inhibition of this modification of eIF-5A coincides with proliferative arrest late in the G1 phase of the cell cycle. This modification occurs in most, if not all, eukaryotes. The present inventors have noted that inhibitors of deoxyhypusyl synthase would be useful in treating diseases associated with unwanted cell proliferation, such as cancer, by blocking the cell cycle.

Brief Summary Text (53):
Parasitic organisms such as Trypanososma cruzi are thought to obtain the polyamines necessary for their growth from their hosts rather than synthesize their own. DFMO

(an ODC inhibitor), decreases the availability of putrescine, a precursor of spermidine and spermine synthesis. DFMO can cure T. brucei infection in mice and is active against African sleeping sickness in humans caused by T. brucei gambiense. DFMO also has clinical utility in Pneumocystis carinii pneumonia and in infection by the coccidian protozoan parasite, Cryptosporidium. In the laboratory, DFMO acts against Acanthamoeba, Leishmania, Giardia, Plasmodia and Eimeria (Marton, L. J. et al., Annu. Rev. Pharmacol. Toxicol. 35:55-91, 1995). Polyamines are also essential for the growth of Hemophilus and Neisseria organisms (Cohen, S. S., A Guide to the Polyamines, Oxford University Press, NY. pp 94-121, 1998). Thus, compounds and methods of the present invention can be used to treat diseases caused by Trypanososma cruzi, T. brucei, Pneumocystis carinii, Cryptosporidium, Acanthamoeba, Leishmania, Giardia, Plasmodia, Eimeria, Hemophilus and Neisseria.

Brief Summary Text (133):
As indicated above, a preferred reporter group is a fluorophore, a chromophore or a luminescer, most preferably dansyl or biotinyl; the polyamine is preferably spermine, spermidine or putrescine. Most preferred for this utilityis monodansylspermine or DACS.

Detailed Description Text (5):
As used herein, the term "polyamine" is intended to mean putrescine, spermine or spermidine, as well as longer linear polyamines, branched polyamines, and the like, which may have between 2 and about 10 nitrogens. Also included in this definition are polyamine derivatives or analogues comprising a basic polyamine chain with any of a number of functional groups bound to a C atom or a terminal or internal N atom. A polyamine derivative may include a terminal linker or spacer group between the polyamine core and a derivatizing function.

Detailed Description Text (32):
The natural polyamines, including putrescine, spermidine and spermine, are incorporated into the compositions of this invention by coupling them to the various "head" and "linker" groups. Other naturally occurring polyamines that can be employed similarly include: N.sup.1 -acetylspermine, N.sup.1 -acetylspermidine, N.sup.8 -acetylspermidine, N.sup.1 -guanidinospermine, cadaverine, aminopropylcadaverine, homospermidine, caldine (norspermidine), 7-hydroxyspermidine, thermine (norspermine), thermospermine, canavalmine, aminopropylhomospermidine, N, N'-bis(3-aminopropyl)cadaverine, aminopentylnorspermidine, N.sup.4 -aminopropylnorspermidine, N.sup.4 -aminopropylspermidine, caldopentamine, homocaldopentamine, N.sup.4 -bis(aminopropyl)norspermidine, thermopentamine, N.sup.4 -bis(aminopropyl)spermidine, caldohexamine, homothermohexamine and homocaldohexamine.

Detailed Description Text (204):
Commercially available amino alcohols 6a are being used in the sequence of steps in FIG. 35. The amino protection, alcohol oxidation, aldehyde protection and amide hydrolysis steps have been successful using 3, 4 and 5-carbon chain amino alcohols 6a. These synthons 8a enable production of polyamine scaffolds mimicking the naturally occurring putrescine, spermidine and spermine.

Detailed Description Text (277):
In each of three 10 ml reaction vials (React-Vial.TM. Pierce, Rockford, Ill.) were placed 0.74 mmol of spermine and 0.15 mmol of triethylamine. Similarly in three additional reaction vials were placed 0.74 mmol of spermine and 0.15 mmol of triethylamine. Similarly in three additional reaction vials were also placed 0.74 mmol of putrescine and 0.15 mmol triethylamine. To each of these flask were added 2.5 ml dry CH.sub.2 Cl.sub.2 and the flasks were closed with a septum and cooled down to -20.degree. C. in a React-block.TM. aluminum block for 45 minutes, when it was placed in a Reacti-Therm.TM. Heating/Stirring Module, with heating switched off. Three acid chlorides (1-naphthylsulfonyl chloride, 2-naphthylsulfonyl chloride and 10-carnphorsulfonyl chloride) in 2.5 ml CH.sub.2 Cl.sub.2 were added dropwise over 15 minutes via a 2.5 ml syringe (All-PP/PE, Aldrich, Milwaukee, Wis.) through the septum to each of spermine and putrescine. Each vial contained also an exhaust consisting of a 2.5 ml syringe filled with anhydrous CaCl.sub.2 with out the plunger. The reactions were allowed to proceed for 16 hours at ambient temperature when it was extracted 2.times.2.5 ml 5% sodium carbonate solution followed by

2.times.2.5 ml water. To the organic solvents were added 2.5 ml methanol and 5 equivalents of a 6N HCl solution. The solvent was evaporated with argon and dried on a high vacuum. Silica gel TLC with isopropanol:acetic acid:pyridine:water 4:1:1:2 showed mainly one component with either UV/fluorescence or 0.2% ninhydrin in ethanol staining. Purity was estimated as to be greater than 80%. The structures, yield and inhibition of the polyamine transporter is shown in Table 1, below.

Detailed Description Text (292):
Screening of thousands of compounds has permitted the present inventors and their colleagues to identify a transport inhibitor that inhibits spermidine uptake with a K.sub.i of 8 nM, putrescine uptake with a K.sub.i of 5.4 nM and has an IC.sub.50 of 0.6 .mu.M for growth in combination with an ODC inhibitor (FIG. 22). Over 100 analogues of this compound have been synthesized and SAR data has been accumulating around the structural features necessary to inhibit polyamine uptake. Additional compounds have been discovered with even greater potency than DACS, but not as exhaustively studied as described below. Under the assay conditions described above, with 1.0 .mu.M supplemented polyamines, there is no growth reduction due to ODC inhibition alone. In addition, DACS is not growth inhibitory alone until very high concentrations (300 .mu.M) are reached. DACS makes the previously ineffective ODC inhibitors very effective as growth inhibitors in the presence of polyamines.

Detailed Description Text (295):
Extracellular spermidine, spermine and putrescine can reverse the effects of ODC inhibitors through increased uptake into the cell. The major excreted forms of polyamines (N.sup.1 -acetylspermine and N.sup.1 -acetylspermidine) can also reverse the effect of ODC inhibitors. DACS prevents the natural polyamines, putrescine, spermidine, N.sup.1 -acetylspermine and N.sup.1 -acetylspermidine, from rescuing the cells from ODC inhibition. This is significant for several reasons. Reports in the literature suggest that there are more than one transporter. If this is true, DACS is effective at blocking the uptake of all of the polyamines at low concentrations.

Other Reference Publication (47):
Hanauske-Abel, H. M. et al., "Detection of a Sub-Set of Polysomal mRNAs Associated with Modulation of Hypusine Formation at the G1-S Boundary Proposal of a Role of eIF-5A in onset of DNA Replication," Febs. Lett., (1995) 366:92-8.

Other Reference Publication (89):
Singh, S. et al., "Characterization of Simian Malarial Parasite (Plasmodium Knowlesi)-induced Putrescine Transport in Rhesus Monkey Erythrocytes," J. Biol. Chem., (1997) 272(21):13506-11.

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L4: Entry 11 of 11 File: USPT Sep 6, 1994

DOCUMENT-IDENTIFIER: US 5344846 A
TITLE: Compositions and methods for inhibiting deoxyhypusine synthase and the growth of cells

Drawing Description Text (7):
FIG. 6 is a graphical illustration of the inhibition pattern for rat testis deoxyhypusine synthase with [.sup.3 H]spermidine as varied substrate and N.sup.1 -guanyl-1,7-diaminoheptane as inhibitor. The level of eIF-5A precursor protein, ec-eIF-5A(Lys), was fixed at 1 .mu.M. The inhibitor concentrations (0.05, 0.03, 0.015, 0.01 or 0.0 .mu.M) are given in .mu.M at the ends of the lines. The initial velocities are given in [.sup.3 H]deoxyhypusine formed (per 6 units of enzyme).

Detailed Description Text (4):
"eIF-5A" refers to eukaryotic translational initiation factor 5A. The nomenclature for initiation factors has been revised (IUB-NC (1989) Eur. J. Biochem 186:1-3). In earlier studies eIF-5A was termed eIF-4D (or IF-M2B.alpha.).

Detailed Description Text (19):
Deoxyhypusine (N.sup..epsilon. -(4-aminobutyl)lysine) is an intermediate in the post-translational biosynthesis of the unique amino acid hypusine (N.sup..epsilon. -(4-amino-2-hydroxybutyl)lysine). Hypusine occurs at a single position in one cellular protein, the eukaryotic translation initiation factor 5A (eIF-5A). eIF-5A, abundant in all eukaryotic cells examined, appears to be essential for protein synthesis and for cell growth. (See, e.g., Cooper et al. (1982) Cell 29:791-797; and Schnier et al. (1991) Mol. Cell. Biol. 11:3105-3114.)

Detailed Description Text (20):
The formation of hypusine occurs in two steps through modification of a lysine residue in the protein precursor of eIF-5A. As shown in FIG. 1, the enzyme deoxyhypusine synthase catalyzes the first step, in which the 4-aminobutyl moiety of the polyamine spermidine is transferred to the .epsilon.-amino group of a specific lysine residue of the precursor to form the deoxyhypusine residue. (See Park et al. (1982) J. Biol. Chem. 257:7217-7222; Park et al. (1988) J. Biol. Chem. 263:15264-15269; and Wolff et al. (1990) J. Biol. Chem. 265:4793-4799.) In the absence of the eIF-5A precursor deoxyhypusine synthase catalyzes the cleavage of spermidine to form .DELTA..sup.1 -pyrroline and 1,3-diaminopropane. The occurrence of this partial reaction suggests that association of spermidine with the enzyme is an early event during catalysis and is independent of binding of the eIF-5A protein precursor.

Detailed Description Text (21):
Deoxyhypusine synthase, purified approximately 700-fold from rat testis, exhibits an apparent molecular mass of 180-190 kDa upon size exclusion chromatography. (See Wolff et al. (1990) J. Biol. Chem. 265:4793-4799.) The K.sub.m(app) values for the substrates, spermidine, NAD.sup.+ and eIF-5A precursor from spermidine-depleted CHO cells, have been calculated as approximately 4, 30, and 0.08 .mu.M, respectively.

Detailed Description Text (22):
The enzyme displays a quite narrow specificity for all of its substrates. Although NADP.sup.+ and NADH were reported to substitute for NAD.sup.+ to some degree with crude enzyme preparations, their effects with the more purified enzyme were found to

be negligible. Neither free lysine nor synthetic 9- or 16-member peptides modeled on the amino acid sequence encompassing the lysine residue in eIF-5A precursors that undergoes modification to hypusine, function as substrates for deoxyhypusine synthase. An eIF-5A precursor, ec-eIF-5A(Lys) [the eIF-5A precursor containing unmodified Lys prepared by over-expression of human eIF-5A cDNA in E. coli], is a good substrate. (See Smit-McBride et al. (1989) J. Biol. Chem. 264:18527-18530; Wolff et al. (1990) J. Biol. Chem. 265:4793-4799; and Park et al. (1991) J. Biol. Chem. 266:7988-7994.) In contrast, an altered eIF-5A precursor protein, in which Lys.sup.50 is replaced by Arg, is not, indicating that only lysine is modified by deoxyhypusine synthase. This finding is consistent with the conclusion that this lysine is the only one in the precursor that is converted to hypusine, both in cells and in the enzyme reaction in vitro.

Detailed Description Text (24):
There is substantial evidence that hypusine, and possibly deoxyhypusine, plays a vital role in the biological activity of eIF-5A. (See, e.g., Park (1989) J. Biol. Chem. 264:18531-18535; Smit-McBride et al. (1989) J. Biol. Chem. 264:18527-18530; and Park et al. (1991) J. Biol. Chem. 266:7988-7994.) Thus, the inhibition of spermidine association with deoxyhypusine synthase would prevent formation of deoxyhypusine, and hence selectively block the production of biologically active forms of eIF-5A protein. This evidence for disruption of the initial step in the post-translational maturation of eukaryotic initiation factor 5A provides a basis for the potential control of protein biosynthesis and cell proliferation.

Detailed Description Text (58):
[1,8-.sup.3 H]Spermidine.multidot.3HCl(20-30 Curies (Ci)/millimoles (mmol)) was purchased from DuPont-New England Nuclear (Boston, Mass.); 1-aminooctane and N.sup.4 -methylcaldine from Aldrich Chemical Company (Milwaukee, Wis.); caldine from Eastman Chemicals (Kingsport, Tenn.); hirudonine-1.5H.sub.2 SO.sub.4 from ICN Biochemicals (Cosa Mesta, Calif.); putreanine.HCl from Vega (Tucson, Ariz.); and N.sup.1 -acetylspermidine.2HCl, N.sup.4 -benzylspermidine, and agmatine.H.sub.2 SO.sub.4 from Sigma (St. Louis, Mo.). N-Butyl-1,3-diaminopropane.2HCl (MDL 73208), N,N'-bisdimethylcaldine.3HCl (MDI 27483), N,N'-bisallylcaldine.3HCl (MDL 27305), N,N'-bisbenzylcaldine.3HCl (MDL 27616), N,N'-bis-benzyl-1,8-diaminooctane.2HCl (MDL 27617), 6-fluorospermidine.3HBr (MDL 72721), 6,6-difluorospermidine.3HBr (MDL 72766) and 7,7-difluorospermidine.3HBr (MDL 72748) were generous gifts from Drs. N. Seiler, P. S. Mamont, and R. Snyder, Merrell Dow Research Institutes of the Marion Merrell Dow Inc. (Strasbourg, France); 5,5-dimethylspermidine.3HCl from Dr. B. Ganem, Cornell University; and 1-methylspermidine.3HCl from Dr. J. K. Coward, University of Michigan. The following compounds were prepared by the referenced procedures: N-(3-aminopropyl) cadaverine.3HCl (Park et al. (1991) J. Biol. Chem. 266:7988-7994); N-(3-cyanopropyl)-1,3-diaminopropane.2HCl (Abbruzzese et al. (1989) Biochem. Biophys. Acta 997:248-255); N-(2-cyanoethyl)-1,4-diaminobutane.2HCl (id.), N.sup.1 N.sup.8 -bis tert butyloxycarbonyl (Boc) spermidine (Nagarajan et al. (1985) J. Org. Chem. 50:5735-5737); N.sup.4 -benzoylspermidine.2F.sub.3 CCOOH (Bergeron et al. (1982) Synthesis 689-692); and N-(3-aminooxypropyl)-1,3-diaminopropane.3HCl (Khomutov, A. R., and Khomutov, R. M. (1989) Bioorg. Khim. 15:698-703). The eIF-5A precursor protein, ec-eIF-5A(Lys) was prepared in the Laboratory of Dr. J. W. B. Hershey, University of California, Davis, Calif., by overexpression of a human eIF-5A cDNA in E. coli as described previously (Smit-McBride et al. (1989) J. Biol. Chem. 264:18527-18530) and further purified on a Mono S column. Deoxyhypusine synthase was prepared from rat testis (Wolff et al. (1990) J. Biol. Chem. 265:4793-4799). The specific activity of the enzyme preparation used was 13,000 units per milligram (mg).

Detailed Description Text (76):
The fluorinated derivatives of spermidine were synthesized and first tested as inhibitors of deoxyhypusine synthase by Annie Bernhardt and Dr. Pierre Mamont of the Marion Merrell Dow Research Institute in Strasbourg, France. Assays were with deoxyhypusine synthase from HTC cells under conditions similar to ours, except that the eIF-5A precursor protein was isolated from DFMO-treated HTC cells and the spermidine level was 1 .mu.M. They found inhibitions of 90%, 86%, 35% and 0% with 6,6-difluorospermidine, 6-monofluorospermidine, 7-monofluorospermidine, and 7,7-difluorospermidine, respectively, at concentrations of 20 .mu.M. The K.sub.i value obtained for 6,6-difluorospermidine was very close to that for

1,3-diaminopropane and to the K.sub.m value for spermidine. There are quantitative differences between these results and the findings given here. However, our observation that the spermidine derivative with fluoride on carbon 7 (compound 38) is devoid of inhibitory activity, whereas those with fluoride on carbon 6 (compounds 36 and 37) are quite strong inhibitors, is in agreement with the earlier findings.

Detailed Description Text (78):
The enzyme assay was performed as previously described (see Wolff et al. (1990) J. Biol. Chem. 265:4793-4799; and Park et al. (1991) J. Biol. Chem. 266:7988-7994). A typical assay mixture included 0.2M glycine, 1 mM dithiothreitol, 0.5 mM NAD.sup.+, 2.4 micromolar (.mu.M) [1,8-.sup.3 H] spermidine, 1 .mu.M ec-eIF-5A(Lys), 25 micrograms (.mu.g) bovine serum albumin, and 3-15 units of enzyme in a total volume of 20 microliters (.mu.l). Incubations were carried out at pH 9.3 and 37.degree. C. for 1 hour. The inhibitors were tested at 0.1 and 1 mM initially, then at lower concentrations as necessary to achieve less than 50% inhibition. Percent inhibition was plotted versus log inhibitor concentration in order to obtain graphic estimates of IC.sub.50 values, i.e., the concentrations inhibiting [.sup.3 H]deoxyhypusine production by 50%. The IC.sub.50 values reported in the tables were calculated by fitting the data points to equation 1 where x=% inhibition and I is the inhibitor concentration. ##EQU1## The nature of the inhibition was determined by plotting reciprocal velocities of [.sup.3 H]deoxyhypusine production against reciprocals of [.sup.3 H]spermidine concentrations at a constant fixed level of ec-eIF-5A(Lys) (1 .mu.M) and at set inhibitor concentrations. With those inhibitors examined, linear plots were obtained and the patterns were indicative of competitive inhibition. Estimates of K.sub.i values were made by fitting the data to equation 2; wherein v is the velocity of the reaction, V is the maximum velocity, S is the substrate concentration, K.sub.m is Michaeli's constant, K.sub.i is the inhibition constant, and I is the inhibitor concentration. ##EQU2## All fits were performed by means of an interactive curve-fitting program, MLAB, developed and modified at the National Institutes of Health to run on an IBM personal computer.

Detailed Description Text (83):
Of the three substrates for deoxyhypusine synthase, spermidine, NAD.sup.+ and eIF-5A precursor protein, the precursor protein is the most specific in the sense that this protein (and specifically a single lysine residue in this protein) is ustilized solely for hypusine production. This is evidenced by the finding that, when CHO cell are depleted of spermidine, eIF-5A precursor protein accumulates. (See Park (1987) J. Biol. Chem. 262:12730-12734 and Park (1988) J. Biol. Chem. 263:7447-7449.) However, a structurally related compound (a 16-membered peptide, modeled on residues 40-55 of the human eIF-5A precursor protein and encompassing the lysine residue, Lys.sup.50, that is modified by deoxyhypusine synthase), was found to exert only weak inhibition and failed to act as a substrate. That this moderate sized peptide reacts only weakly, if at all, with the enzyme was seen as evidence that macromolecular features, rather than simple structural similarity, may be necessary for precursor protein-enzyme interaction.

Detailed Description Text (85):
The efficiency of di- and polyamines as inhibitors of the enzyme varied depending on the number of methylene groups between their primary amino groups. With the exception of 1,3-diaminopropane (compound 2), which exerts product inhibition, and to a lesser extent putrescine (compound 3), the inhibition was maximal with compounds that resemble spermidine in carbon chain length. The presence of a secondary amino group in the molecule did not seem to be important as evidenced by the strong inhibition with 1,7-diaminoheptane (compound 6) and 1,8-diaminooctane (compound 7). In an extended conformation in which their amino groups are as far apart as possible, these two compounds resemble the fully extended form of spermidine in distance between primary amino groups, compound 6 being slightly shorter and compound 7 a bit longer. A similar relationship exists with caldine (compound 9) and N-(3-aminopropyl)cadaverine (compound 11), respectively, both of which provide some degree of inhibition. Interestingly, N-(3-aminopropyl)cadaverine was found to function as a substrate for deoxyhypusine synthase, albeit a poor one (see Park et al. (1991) J. Biol Chem. 266:7988-7994). Under the same conditions, caldine, however, displayed no detectable substrate property. The failure of spermine (compound 12) to function as an inhibitor may be a consequence of the relatively great distance between its primary amino groups.

Detailed Description Text (88):
Parenthetically, it is not surprising that 1,3-diaminopropane (compound 2) and putrescine (compound 3) inhibit the enzyme, since the former is a product of the reaction (see Park et al. (1988) J. Biol. Chem. 263:15264-15269) and the latter, being of similar structure, may inhibit by interaction at the product site. It may be significant, however, that the inhibition by diaminopropane is competitive. When spermidine binds to the enzyme through its primary amino groups, it assumes a less than fully extended conformation in which its secondary amino group, which is not essential for binding, is directed, through precise alignment of its backbone structure, to the optimum position for catalysis. Those compounds of FIG. 3 that are closely related in structure to spermidine, with the exception of having a single primary amino group (compounds 14-17) or one or both primary amino groups derivatized (compounds 18-21), show no inhibition.

Detailed Description Text (103):
The mild degree of inhibition exerted by agmatine (compound 47) is not inconsistent with this supposition since its interaction could occur at the position on the enzyme in which the butylamine moiety of spermidine is normally arranged, i.e., with its guanidino group directed toward the site that favors this group over the amino group. It follows from this suggestion, incidentally, that putrescine and 1,3-diaminopropane inhibit by binding at different positions on the enzyme, each occupying a separate portion of the spermidine-binding site. That N.sup.8 -guanylspermidine (compound 52) is a somewhat more effective inhibitor than N.sup.1 -guanylspermidine (compound 51) is in accordance with an idea that because of the preferences and arrangement of the two binding sites for the primary amino groups of spermidine, this polyamine and its N.sup.8 -guanyl derivative attach to enzyme in a similar fashion. However, despite its high affinity for the enzyme, the spermidine derivative apparently is not aligned on the enzyme in exactly the same way as is spermidine. If it were, it would function as a substrate which it does not. Perhaps the difference in alignment on the enzyme is due to the greater size of the guanidinium group and/or its tendency to form multiple zwitterionic hydrogen bonds with anionic ligands (see, e.g. Dietrich et al. (1979) Helv. Chim. Acta 62:2763-2787; and Makhatadze et al. (1992) J. Mol. Biol. 226:491-505), which may restrict the conformation of either the guanidinium compound or the protein.

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**Search Results** - Record(s) 11 through 11 of 11 returned.

☑ 11. Document ID: US 5344846 A

L4: Entry 11 of 11 File: USPT Sep 6, 1994

US-PAT-NO: 5344846
DOCUMENT-IDENTIFIER: US 5344846 A

TITLE: Compositions and methods for inhibiting deoxyhypusine synthase and the growth of cells


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| Term | Documents |
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| EIF-5A.DWPI,EPAB,JPAB,USPT,PGPB. | 36 |
| EIF-5AS | 0 |
| (EIF-5A AND 3).USPT,PGPB,JPAB,EPAB,DWPI. | 11 |
| (L3 AND EIF-5A).USPT,PGPB,JPAB,EPAB,DWPI. | 11 |

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**Search Results** - Record(s) 1 through 10 of 12 returned.

1. Document ID: US 20020160403 A1

L8: Entry 1 of 12 File: PGPB Oct 31, 2002

PGPUB-DOCUMENT-NUMBER: 20020160403
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020160403 A1

TITLE: huBUB3 gene involved in human cancers

PUBLICATION-DATE: October 31, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Seeley, Todd W. | Moraga | CA | US | |

US-CL-CURRENT: 435/6; 435/226, 435/320.1, 435/325, 435/69.1, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

2. Document ID: US 20020123042 A1

L8: Entry 2 of 12 File: PGPB Sep 5, 2002

PGPUB-DOCUMENT-NUMBER: 20020123042
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020123042 A1

TITLE: DETECTION OF LOSS OF THE WILD-TYPE HUBUB1 GENE

PUBLICATION-DATE: September 5, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| SEELEY, TODD W. | MORAGA | CA | US | |

US-CL-CURRENT: 435/6; 435/320.1, 435/325, 435/455, 435/69.1, 536/23.1, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

3. Document ID: US 20020055625 A1

L8: Entry 3 of 12 File: PGPB May 9, 2002

PGPUB-DOCUMENT-NUMBER: 20020055625
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020055625 A1

TITLE: Members of TNF and TNFR families

PUBLICATION-DATE: May 9, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Tribouley, Catherine | San Francisco | CA | US | |

US-CL-CURRENT: 536/23.5; 435/4, 530/351

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

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☐ 4. Document ID: US 20020045663 A1

L8: Entry 4 of 12 File: PGPB Apr 18, 2002

PGPUB-DOCUMENT-NUMBER: 20020045663
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020045663 A1

TITLE: D-enantiomer of DFMO and methods of use therefor

PUBLICATION-DATE: April 18, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Levenson, Corey | San Antonio | TX | US | |
| Shaked, Ze?apos;ev | Boston | MA | US | |

US-CL-CURRENT: 514/564

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

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☐ 5. Document ID: US 20020009739 A1

L8: Entry 5 of 12 File: PGPB Jan 24, 2002

PGPUB-DOCUMENT-NUMBER: 20020009739
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020009739 A1

TITLE: Metastatic breast and colon cancer regulated genes

PUBLICATION-DATE: January 24, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Giese, Klaus | Berlin | | DE | |

US-CL-CURRENT: 435/6; 435/183, 435/7.23, 530/388.8

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

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☐ 6. Document ID: US 6468790 B1

L8: Entry 6 of 12 File: USPT Oct 22, 2002

US-PAT-NO: 6468790
DOCUMENT-IDENTIFIER: US 6468790 B1

TITLE: Metastatic breast and colon cancer regulated genes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

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☐ 7. Document ID: US 6432668 B1

L8: Entry 7 of 12 File: USPT Aug 13, 2002

US-PAT-NO: 6432668
DOCUMENT-IDENTIFIER: US 6432668 B1

TITLE: Polynucleotides encoding human cyclin-dependent kinase (hPFTAIRE)

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

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☐ 8. Document ID: US 6410312 B1

L8: Entry 8 of 12 File: USPT Jun 25, 2002

US-PAT-NO: 6410312
DOCUMENT-IDENTIFIER: US 6410312 B1

TITLE: huBUB3 gene involved in human cancers

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

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☐ 9. Document ID: US 6391899 B1

L8: Entry 9 of 12 File: USPT May 21, 2002

US-PAT-NO: 6391899
DOCUMENT-IDENTIFIER: US 6391899 B1

TITLE: Compounds and compositions for treating tissue ischemia

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

10. Document ID: US 6368598 B1

L8: Entry 10 of 12 File: USPT Apr 9, 2002

US-PAT-NO: 6368598
DOCUMENT-IDENTIFIER: US 6368598 B1

TITLE: Drug complex for treatment of metastatic prostate cancer

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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| DELIVER$ | 0 |
| DELIVER.DWPI,EPAB,JPAB,USPT,PGPB. | 224979 |
| DELIVERA.DWPI,EPAB,JPAB,USPT,PGPB. | 1 |
| DELIVERAB.DWPI,EPAB,JPAB,USPT,PGPB. | 1 |
| DELIVERABILITIES.DWPI,EPAB,JPAB,USPT,PGPB. | 3 |
| DELIVERABILITY.DWPI,EPAB,JPAB,USPT,PGPB. | 282 |
| "DELIVERABILITY-I.E".DWPI,EPAB,JPAB,USPT,PGPB. | 1 |
| DELIVERABILY.DWPI,EPAB,JPAB,USPT,PGPB. | 1 |
| DELIVERABL.DWPI,EPAB,JPAB,USPT,PGPB. | 1 |
| (L6 AND (PUTRESCINE SAME (DELIVER$ OR ADMINISTER$))).USPT,PGPB,JPAB,EPAB,DWPI. | 12 |

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**Search Results** - Record(s) 11 through 12 of 12 returned.

☐ 11. Document ID: US 6297367 B1

L8: Entry 11 of 12 File: USPT Oct 2, 2001

US-PAT-NO: 6297367
DOCUMENT-IDENTIFIER: US 6297367 B1

TITLE: Polynucleotide encoding TNFL1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 12. Document ID: US 6258845 B1

L8: Entry 12 of 12 File: USPT Jul 10, 2001

US-PAT-NO: 6258845
DOCUMENT-IDENTIFIER: US 6258845 B1

TITLE: DFMO and sulindac combination in cancer chemoprevention

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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| Term | Documents |
|---|---|
| PUTRESCINE.DWPI,EPAB,JPAB,USPT,PGPB. | 1303 |
| PUTRESCINES.DWPI,EPAB,JPAB,USPT,PGPB. | 1 |
| DELIVER$ | 0 |
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| DELIVERABILITY.DWPI,EPAB,JPAB,USPT,PGPB. | 282 |
| "DELIVERABILITY-I.E".DWPI,EPAB,JPAB,USPT,PGPB. | 1 |
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| DELIVERABL.DWPI,EPAB,JPAB,USPT,PGPB. | 1 |
| (L6 AND (PUTRESCINE SAME (DELIVER$ OR ADMINISTER$))).USPT,PGPB,JPAB,EPAB,DWPI. | 12 |

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L8: Entry 12 of 12 File: USPT Jul 10, 2001

DOCUMENT-IDENTIFIER: US 6258845 B1
TITLE: DFMO and sulindac combination in cancer chemoprevention

Abstract Text (1):
Activation of the Ki-ras proto-oncogene is common in colon carcinogenesis. Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit carcinogen-induced colon carcinogenesis, decrease the frequency of Ki-ras mutations in the azoxymethane-treated rat model, and induce apoptosis in a variety of cell types. Sulindac, as well as other non-steroidal anti-inflammatory agents, are provided in combination with DFMO the prevention and/or treatment of cancers characterized by the expression of an activated Ki-ras. Provided with the present invention are pharmaceutically acceptable compositions that include a non-steroidal anti-inflammatory agent, sulindac, together with an effective amount of difluoromethylornithine.

Brief Summary Text (5):
Mutational activation of the Kirsten (Ki)-ras oncogene is an important genetic alteration in colorectal neoplasia. Ki-ras mutations have been detected in approximately 50 percent of sporadic human colorectal tumors (Vogelstein et al., 1988; Burmer and Loeb, 1989). Ki-ras mutations have been detected in aberrant crypt foci, as well as in adjacent regions of histologically normal mucosa (Losi et al., 1996). These findings suggest that the mutation of Ki-ras may be a relatively early event in the temporal development of colon cancer. Ki-ras also is mutated in chemically-induced rodent tumors, such as the azoxymethane (AOM)-treated rat model, with a frequency similar to that of human cancers (Erdman, 1997; Vivona et al., 1993). Although the role of Ki-ras in tumorigenesis is unclear, activation of this gene has been correlated with deficient apoptosis in human colorectal neoplasms (Ward et al., 1997).

Brief Summary Text (6):
The nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin, ibuprofen, piroxicam (Reddy et al., 1990; Singh et al., 1994), indomethacin (Narisawa, 1981), and sulindac (Piazza et al., 1997; Rao et al., 1995), effectively inhibit colon carcinogenesis in the AOM-treated rat model. NSAIDs also inhibit the development of tumors harboring an activated Ki-ras (Singh and Reddy, 1995). NSAIDs appear to inhibit carcinogenesis via the induction of apoptosis in tumor cells (Bedi et al., 1995; Lupulescu, 1996; Piazza et al., 1995; Piazza et al., 1997b). A number of studies suggest that the chemopreventive properties of the NSAIDs, including the induction of apoptosis, is a function of their ability to inhibit prostaglandin synthesis (reviewed in DuBois et al., 1996; Lupulescu, 1996; Vane and Botting, 1997). Recent studies, however, indicate that NSAIDs may act through both prostaglandin-dependent and -independent mechanisms (Alberts et al., 1995; Piazza et al., 1997a; Thompson et al., 1995; Hanif, 1996). Sulindac sulfone, a metabolite of the NSAID sulindac, lacks COX-inhibitory activity yet induces apoptosis in tumor cells (Piazza et al., 1995; Piazza et al., 1997b) and inhibits tumor development in several rodent models of carcinogenesis (Thompson et al., 1995; Piazza et al., 1995, 1997a).

Brief Summary Text (9):
The importance of Ki-ras activation in NSAID-mediated chemoprevention has not yet been determined. NSAIDs induce apoptosis in both colon tumor cell lines and animal

tissues, and appear to inhibit Ki-ras activation in tumors, however the activation of Ki-ras has not yet been investigated as a mechanism of NSAID-mediated cytotoxicity. It also is not known if such cytotoxicity is dependent on the anti-inflammatory properties of the NSAIDs. The NSAID sulindac, which also inhibits Ki-ras activation, is metabolized to two different molecules which differ in their ability to inhibit COX, yet both are able to exert chemopreventive effects via the induction of apoptosis. Sulindac sulfone lacks COX-inhibitory activity, and most likely facilitates the induction of apoptosis in a manner independent of prostaglandin synthesis.

Brief Summary Text (10):
.alpha.-Difluoromethylornithine (DFMO) is an enzyme-activated, irreversible inhibitor of ornithine decarboxylase (ODC) and causes depletion in the intracellular concentrations of putrescine and its derivative, spermidine (Pegg, 1988). Levels of spermine, which is derived from spermidine, are not as markedly affected by the enzyme inhibition. DFMO was initially synthesized for therapeutic anticancer usage, but it was found not to be an active cytotoxic agent in chemotherapy trials against human cancer (McCann and Pegg, 1992), except perhaps demonstrating moderate activity in the treatment of malignant brain tumors (Levin et al., 1987). In general, the compound was nontoxic, with the significant exception of hearing loss, which was reversible after the drug treatment was discontinued (Meyskens et al., 1986). The onset of the hearing loss could be associated with total cumulative dose (Croghan et al., 1991).

Brief Summary Text (11):
In experimental animal models, DFMO is a potent inhibitor of carcinogenesis that is especially active in preventing carcinogen-induced epithelial cancers of many organs, including those of the colon (Weeks et al., 1982; Thompson et al., 1985; Nowels et al., 1986; Nigro et al., 1987). DFMO acts late in the tumor-promotion phase in animals, but the precise mechanism by which it inhibits the development of polyps and cancers is unknown. Effects on cell transformation, invasion, and angiogenesis by ornithine decarboxylase and polyamines have been reported (Auvinen, 1997); for example, overexpression of ODC enhances cellular transformation and invasion (Kubota et al., 1997).

Drawing Description Text (3):
FIG. 1. Ki-ras transfection of Caco-2 human colon adenocarcinoma cells. Caco-2 cells were transfected with a plasmid directing the overexpression of an activated Kirsten-ras gene and subject to drug-resistance selection and clone. Detection of Ki-ras expression in various transfected clones by western blot, utilizing an antibody which detects both normal and activated p21.sup.K-ras K. Numbered lanes refer to clonal isolates of transfected cells; Caco-2, untransfected parental cells; Caco-2/Ki-ras, pooled clones.

Drawing Description Text (4):
FIG. 2. Effect of sulindac sulfide and sulfone on the growth of Ki-ras transfected cells. Caco-2 parental cells (.smallcircle.), Ki-ras clone #60 (.quadrature.), and Ki-ras clone #66 (.DELTA.) were seeded in the presence of vehicle (A), 120 .mu.M sulindac sulfide (B), or 600 .mu.M sulindac sulfone (C) and harvested at 2-day intervals. Viable cell number was determined by trypan blue dye exclusion.

Drawing Description Text (5):
FIG. 3. Induction of apoptosis by sulindac sulfide and sulfone in Ki-ras-transfected cells. Caco-2 parental cells (.smallcircle.), Ki-ras clone #60 (.quadrature.), and Ki-ras clone #66 (.DELTA.) were seeded in the presence of vehicle (A), 120 .mu.M sulindac sulfide (B), or 600 .mu.M sulindac sulfone (C) and harvested at 2-day intervals. Apoptosis was determined by light microscopy.

Drawing Description Text (6):
FIG. 4. Survival of Caco-2 parental or Ki-ras clone #60 cells in sulfide or sulfone. Caco-2 parental cells (.smallcircle.) and Ki-ras clone #60 (.quadrature.) were treated for 24 h with increasing concentrations of sulindac sulfide (0, 75, 150, and 300 .mu.M) (A) or sulindac sulfone (0, 300, 600 and 1200 .mu.M) (B). Cells were replated at diluted concentrations in the absence of drug and grown for 21 days. Plates were stained with crystal violet and the number of colonies on each plate was

determined. Plating efficiency was determined by dividing the number of colonies by the total number of cells plated. These numbers were then normalized to the plating efficiency of non-drug-treated controls of the same line. Data points without error bars contain a standard deviation too small to be represented.

Drawing Description Text (7):
FIG. 5. Effect of DFMO on survival of Caco-2 parental or Ki-ras clone #60 cells in sulindac sulfide or sulfone. Caco-2 parental (.circle-solid.) or Ki-ras clone #60 (.box-solid.) cells were treated for 24 h with 5 mM DFMO, and then treated for an additional 24 h with increasing concentrations of sulindac sulfide (0, 75,150, and 300 mM) (A) or sulindac sulfone (0, 300, 600, 1200 mmM) (B) in the absence of DFMO. Cells were replated at diluted concentrations and grown for 21 days. Plates were stained with crystal violet and the number of colonies on each plate was determined. Plating efficiency was determined by dividing the number of colonies by the total number of cells plated. These numbers were then normalized to the plating efficiency of non-drug-treated controls of the same line. Data points without error bars contain a standard deviation too small to be represented.

Drawing Description Text (8):
FIG. 6. Effect of DFMO on the dose-dependent toxicity of sulindac sulfide or sulfone. Caco-2 parental (.smallcircle.,.circle-solid.), or Ki-ras #60 cells (.quadrature.,.box-solid.) were treated with vehicle (.smallcircle.,.quadrature.) or 5 mM difluoromethylomithine (DFMO) (.circle-solid.,.box-solid.) for 24 h, and then treated for an additional 24 h with increasing concentrations of sulindac sulfide (0, 75, 150, and 300 .mu.M) (A) or sulindac sulfone (0, 300, 600, and 1200 .mu.M) (B), in the absence of DFMO. Cells were replated at diluted concentrations and grown for 21 days. Plates were stained with crystal violet and the number of colonies on each plate was determined. Plating efficiency was determined by dividing the number of colonies by the total number of cells plated. These values were then normalized to the plating efficiency of control cells that were treated similarly with respect to DFMO dosage. Data points without error bars contain a standard deviation too small to be represented.

Drawing Description Text (9):
FIG. 7. Effect of DFMO on survival of Caco-2 and Ki-ras transfected cells. Caco-2 (.circle-solid.), Ki-ras #60 (.box-solid.) or Ki-ras #66 (.tangle-solidup.) cells were treated with 0, 50, 500, and 5000 .mu.M DFMO for 24 h prior to subsequent dilution and reseeding in drug-free media Cells were grown for approximately 21 days and then analyzed for colony-forming efficiency. Plating efficiency was determined by dividing the number of colonies by the total number of cells plated. These values were then normalized to the plating efficiency of untreated control cells of like cell type. Data points without error bars contain a standard deviation too small to be represented.

Drawing Description Text (10):
FIG. 8. The effect of DFMO on apoptosis induced by sulindac sulfone in Caco-2 parental or Ki-ras transfected cells. Caco-2 cells (8A) or Caco-2 Ki-ras clone 60 (8B) were seeded in the presence or absence of 5 mM .alpha.-difluoromethylomithine (DFMO) with or without 600 mM sulindac sulfone (SN). Cells were grown over a period of 6 days. Cells were harvested at various days (0, 2, 4 and 6 days), spun onto slides and stained. Apoptosis was determined by light microscopy. Percentage of apoptotic cells was calculated as the number of apoptotic cells out of the total cells counted times 100. At least 500 cells were counted for each sample. In FIG. 8A, -.smallcircle.-=Caco-2+DFMO, -.DELTA.-=Caco-2+SN, and -.circle-solid.-=Caco-2+DFMO, SN. In FIG. 8B, -.smallcircle.-=#60+DFMO, -.DELTA.-=#60+SN, and -.box-solid.-=#60+DFMO, SN.

Detailed Description Text (2):
There is a need for effective and less toxic methods for preventing and/or treating cancers. Current treatment protocols, especially those for colon cancers and polyps, includes tumor resection, chemotherapy and radiation therapy. Colorectal cancer is the second leading cause of death from cancer in The United States. During the last fifteen years, emphasis has been placed on identification of high risk patients and families and outline of appropriate surveillance regimens for normal and high risk patients for colorectal cancer. The present invention concerns the development of an

effective and safe drug combination that will improve the prognosis of certain cancers. The present invention provides for the use of two inhibitors of colon carcinogenesis, DFMO and sulindac, which affect the same ras-dependent signaling pathway. When this pathway is disrupted, carcinogenesis is disrupted, leading to inhibiting the growth of the cancer cells, killing the cancer cell outright, inducing apoptosis, inhibiting metastasis, reducing overall tumor burden, inducing tumor regression, or any combination of these. This complementary action in the same pathway makes this combination more potent than either drug alone and better tolerated than either drug alone. This drug combination also serves to resolve a major problem in cancer, which is to prevent or effectively treat cancers without causing undue toxicity to patients.

Detailed Description Text (4):
The present invention involves the delivery of therapeutic compounds to individuals to reduce or inhibit cancer cells. Target cancer cells include cancers of the lung, brain, prostate, kidney, liver, ovary, breast, skin, stomach, esophagus, head and neck, testicles, colon, cervix, lymphatic system and blood. Of particular interest are epithelial cancers of many organs, including those of the colon and polyps which tend to express an activated Ki-ras.

Detailed Description Text (5):
The present invention also involves the delivery of therapeutic compounds to individuals exhibitting pre-cancerous symptoms to prevent the onset of cancer. Cells of this catagory include polyps and other precancerous lesions, premalignancies, preneoplastic or other abarent phenotype indicating probable progression to a cancerous state.

Detailed Description Text (7):
Ras defines a protooncogene product that is found on chromosome 11. It is found in normal cells, where it helps to relay signals by acting as a switch (Lowy and Willumsen, 1993). When receptors on the cell surface are stimulated (by a hormone, for example), Ras is switched on and transduces signals that tell the cell to grow. If the cell-surface receptor is not stimulated, Ras is not activated and so the pathway that results in cell growth is not initiated. In about 30% of human cancers, Ras is mutated so that it is permanently switched on, telling the cell to grow regardless of whether receptors on the cell surface are activated or not. Point mutations in the cellular ras gene (c-ras) also can result in a mutant p21 protein that can transform mammalian cells.

Detailed Description Text (8):
Ras is a family of retrovirus-associated DNA sequences originally isolated from Harvey (H-ras, Ha-ras, rasH) and Kirsten (K-ras, Ki-ras, rasK) murine sarcoma viruses. Ras genes are widely conserved among animal species and sequences corresponding to both H-ras and K-ras genes have been detected in human, avian, murine, and non-vertebrate genomes. The closely related N-ras gene has been detected in human neuroblastoma and sarcoma cell lines. All genes of the family have a similar exon-intron structure and each encodes a p21 protein

Detailed Description Text (10):
Familial Adenomatous Polyposis (FAP), an inherited polyposis syndrome, is the result of germ-line mutation of the adenomatous polyposis coli (APC) tumor suppresor gene (Su et al., 1992). This autosomal-dominant condition with variable expression is associated with the development of hundreds of colonic adenomas, which uniformly progress to adenocarcinoma by forty years of age, two decades earlier than the mean age diagnosis for sporadic colon cancer (Bussey, 1990). In prior studies of pre-symptomatic individuals with FAP, increased levels of the polyamines spermidine and spermine, and their diamine precursor putrescine, have been detected in normal-appearing colorectal biopsies when compared to normal family member controls (Giardiello et al., 1997). The activity of ornithine decarboxylase (ODC), the first and rate-limiting enzyme in mammalian polyamine synthesis, also is elevated in apparently normal colonic mucosal biopsies from FAP patients (Giardiello et al., 1997; Luk and Baylin, 1984). These findings are of interest as the polyamines are necessary for optimal cell proliferation (Pegg, 1986). Further, suppression of ODC activity, using the enzyme-activated irreversible inhibitor DFMO, inhibits colon carcinogenesis in carcinogen-treated rodents (Kingsnorth et al., 1983; Tempero et

al., 1989).

Detailed Description Text (14):
In recent years, chemotherapeutic agents that directly inhibit polyamine synthesis have been developed. Difluoromethylomithine (DFMO), one such drug, is an irreversible inhibitor of ODC and potentially can be given continuously with significant anti-tumor effects. This drug is relatively non-toxic at low doses of 0.4 gr/M.sup.2 /day to humans while producing inhibition of putrescine synthesis in tumors. Studies in a rat-tumor model demonstrate that DMFO infusion can produce a 90% decrease in tumor putrescine levels without suppressing peripheral platelet counts.

Detailed Description Text (16):
Although DFMO can effectively block tumor putrescine biosynthesis, the resultant antitumor effect is cytostasis and not cytotoxicity. For example, DFMO reduces the growth rate of an MCA sarcoma but does not produce tumor regression. This finding is consistent with reports of other investigators who showed that DFMO is a cytostatic agent. However, studies indicate that a significant role exists for DFMO agents, permitting the future development of combination chemotherapeutic regimens which incorporate DFMO.

Detailed Description Text (17):
DFMO and its use in the treatment of benign prostatic hypertrophy are described in two patents, U.S. Pat. Nos. 4,413,141, and 4,330,559. U.S. Pat. No. 4,413,141 describes DFMO as being a powerful inhibitor of ODC, both in vitro and in vivo. Administration of DFMO causes a decrease in putrescine and spermidine concentrations in cells in which these polyamines are normally actively produced. Additionally, DFMO has been shown to be capable of slowing neoplastic cell proliferation when tested in standard tumor models. U.S. Pat. No. 4,330,559 describes the use of DFMO and DFMO derivatives for the treatment of benign prostatic hypertrophy. Benign prostatic hypertrophy, like many disease states characterized by rapid cell proliferation, is accompanied by abnormal elevation of polyamine concentrations. The treatment described within this reference can be administered to a patient either orally, or parenterally.

Detailed Description Text (18):
The initial promise of DFMO as a therapeutic ODC inhibitor for use in the treatment of various neoplasias has dimmed somewhat because, although DFMO does, in fact, irreversibly inhibit ODC activity, cells treated in vivo with DFMO significantly increase their uptake of exogenous putrescine as described in U.S. Pat. No. 4,925,835. The intercellular transport mechanisms of the cell do an "end run" around the DFMO-impaired ODC activity by importing putrescine from the extra-cellular milieu. Therefore, DFMO's effect in vivo is far poorer than in vitro. So, while DFMO treatment effectively inhibits intracellular putrescine neogenesis, it also results in increased uptake of extracellular putrescine, thereby offsetting its ODC inhibitory effect.

Detailed Description Text (19):
This problem is compounded by the fact that putrescine is present in many common foods, such as orange juice, which contains approximately 400 ppm putrescine. This makes it virtually impossible to provide a patient a nutritionally sufficient diet which is free of putrescine. Therefore, DFMO-treated cells are capable of importing sufficient amounts of extracellular putrescine to support cell division.

Detailed Description Text (21):
However, because DFMO is an effective inhibitor of ODC, some researchers are attempting to use DFMO as part of a conjunctive treatment in combination with other therapeutic agents. For instance, U.S. Pat. No. 4,499,072, describe improving the polyamine-depletion effects of ODC inhibitors (including DFMO) by using interferon in combination with the ODC inhibitor. Additionally, it describes the use of both an ODC inhibitor and interferon in conjunction with a known cytotoxic agent such as methotrexate. U.S. Pat. No. 5,002,879, describe a similar conjunctive therapy in which an ODC inhibitor, preferably DFMO, is used in combination with lymphokine-activated killer (LAK) cells and interleukin-2.

Detailed Description Text (24):
NSAIDs are anti-inflammatory agents that are not steroids. In addition to anti-inflammatory actions, they have analgesic, antipyretic, and platelet-inhibitory actions. They are used primarily in the treatment of chronic arthritic conditions and certain soft tissue disorders associated with pain and inflammation. They act by blocking the synthesis of prostaglandins by inhibiting cyclooxygenase, which converts arachidonic acid to cyclic endoperoxides, precursors of prostaglandins. Inhibition of prostaglandin synthesis accounts for their analgesic, antipyretic, and platelet-inhibitory actions; other mechanisms may contribute to their anti-inflammatory effects. Certain NSAIDs also may inhibit lipoxygenase enzymes or phospholipase C or may modulate T-cell function. (AMA Drug Evaluations Annual, 1994, p 1814-5)

Detailed Description Text (42):
Results of the therapeutic treatments described above using the combination of DFMO and sulindac on patients with tumors can vary. The therapy may inhibit the growth of the cancer cells, kill the cancer cell outright, induce apoptosis, inhibit metastasis, reduce overall tumor burden, induce tumor regression, or any combination of these. Any and all of these results are adventageous to the patient.

Detailed Description Text (48):
Where clinical application of liposomes containing therapeutic compounds is undertaken, it will be necessary to prepare the liposome complex as a pharmaceutical composition appropriate for the intended application. Generally, this will entail preparing a pharmaceutical composition that is essentially free of pyrogens, as well as any other impurities that could be harmful to humans or animals. One also will generally desire to employ appropriate buffers to render the complex stable and allow for uptake by target cells.

Detailed Description Text (56):
Cell culture and drug treatments

Detailed Description Text (57):
Caco-2 cells were obtained from the American Type Culture Collection (Rockville, Md.). Caco-2 cells were maintained in Modified Eagle Media (GIBCO, Grand Island, N.Y.) supplemented with 10% fetal bovine serum and a 1% penicillin (10,000 units/ml)-streptomycin (10,000 mg/ml) solution (GIBCO, Grand Island, N.Y.). Transfected Caco-2 cells resistant to neomycin were maintained in 400 .mu.g/ml G418. Cells were maintained at 37.degree. in the presence of 5% CO.sub.2 in air.

Detailed Description Text (58):
All cells were passed into new medium every two to three days, just prior to confluence. Drug additions were performed at the time of cell seeding and in MEM for each cell line unless otherwise noted. DFMO was provided by Marion Merrel Dow Company (Cincinnati, Ohio). The two metabolites of sulindac, sulindac sulfone and sulfide, were obtained from Cell Pathways, Inc. (Horsham Pa.).

Detailed Description Text (59):
Cell number and viability determinations

Detailed Description Text (60):
Caco-2 cells were removed from the monolayer by treatment with trypsin (1500 units/ml, Calbiochem, San Diego, Calif.)-EDTA (.7mM) and counted using a hemocytometer. A sample of the cell suspension was combined in a 1:1 volume ratio with trypan blue dye (GIBCO, Grand Island, N.Y.), and at least two independently prepared suspensions were counted on a hemocytometer, two counts each. For all cell types, viability was determined by the percentage of cells able to exclude the trypan blue dye.

Detailed Description Text (61):
Apoptosis Quantitation

Detailed Description Text (62):
Apoptosis was quantitated by morphological examination of cells on prepared slides. Each sample was prepared by pooling trypsinized cells with the aspirated culture

medium and an additional saline wash of the tissue culture plate. Slides for light microscopy were prepared by placing 20,000 or 40,000 cells into a cytospin cup and pelleting the cells onto slides via centrifugation using a cytospin (Shandon Lipshaw, Pittsburgh, Pa.) at 600 r.p.m. for 2 min. Cells were affixed to the slides by immersion of the slide into 100% methanol for at least 1 min. Slides were stained using a 1:10 (for CHO cells) or a 1:20 (for Caco-2 cells) dilution of Modified Giemsa stain (Sigma Chemical Co., St. Louis, Mo.). Apoptotic cells were identified by characteristic chromatin condensation, cytoplasmic vacuole formation, cell shrinkage, and formation of apoptotic "bodies" (Kerr et al., 1994). Apoptotic bodies which were not enclosed by membranes were not included in the cell scoring. At least 500 cells were scored for each treatment for each day and frequencies were expressed as a percentage of the total cells counted.

Detailed Description Text (64):
Caco-2 cell survival was determined by colony-forming efficiency. Caco-2 cells or the Ki-ras-transfected clones were plated at a cell density of 2.times.10.sup.5 cells per 60 mm dish in MEM, in the presence or absence of DFMO at varied concentrations. Cells were grown for 24 h, and then the DFMO media was removed. The plates were rinsed twice with saline, and MEM containing varied concentrations of sulindac sulfide or sulfone was added. Cells were grown for an additional 24 h, and then replated at 3 serially diluted concentrations, with 3 plates seeded for each concentration. Approximately 21 days later, the plates were stained for colony formation.

Detailed Description Text (65):
Plates were removed from the incubator and an equal volume of a 3:1 volume ratio of methanol and acetic acid was added to each plate for 5 min. The supernate was aspirated, and the colonies were stained by the addition of approximately 1 mL of crystal violet stain (5 mg/mL in 100% ethanol) (Sigma Chemical Co., St. Louis, Mo.). The plates were then rinsed in distilled water and air dried. Colonies were defined as consisting of a minimum of 50 cells, with each plate containing 20 or more colonies for inclusion in the data set. Standard deviations were prepared from data in which all 3 plates contained a sufficient number of colonies to be included.

Detailed Description Text (67):
A cDNA encoding an activated Ki-ras.sup.Val12 was purchased from the American Type Culture Collection (Rockville, Md.). This cDNA was ligated into the multiple cloning site of a pCDNA3 mammalian expression vector (Invitrogen Corp., Carlsbad, Calif.), and vectors containing the Ki-ras insert were isolated according to standard protocols (Ausubel, 1995). Large-scale amounts of plasmid (mg quantities) suitable for mammalian cell transfection were purified using the Nucleobond Plasmid Kit (Clontech Laboratories, Inc., Palo Alto, Calif.).

Detailed Description Text (68):
The calcium phosphate transfection method was used to introduce the pCDNA3-Ki-ras plasmid into Caco-2 cells, according to established protocols (Ausubel, 1995). One week after transfection, geneticin (Life Technologies, Inc., Germantown, Md.) was added to the media at a concentration of 400 .mu.g/ml. Stable clones were isolated with the use of trypsin soaked filter disks placed over isolated colonies on a tissue culture plate, and then transferred to the wells of a 48-well plate. Clones were maintained in 400 .mu.g/ml of geneticin, and screened via western blot for Ki-ras expression.

Detailed Description Text (69):
Preparation of whole cell lysates of Caco-2 cells

Detailed Description Text (70):
Whole-cell lysates of Caco-2 cells were prepared according to commercially available protocols (Santa Cruz Biotechnology, Santa Cruz, Calif.). Plates were kept on ice, and cells were scraped off the tissue culture plates in the presence of RIPA buffer (PBS, 1% NP-40, .5% sodium deoxycholate, 0.1% sodium dodecylsulfate, 10 mg/ml PMSF, 30 .mu.g/ml aprotinin, 100 mM sodium orthovanadate). The cell suspension was then passed twice through a 22-gauge needle, and was centrifuged at 10000.times.g for 20 min to clarify the lysate.

Detailed Description Text (78):
Isolation and characterization of Caco-2 transfected cells

Detailed Description Text (79):
Caco-2 human colon adenocarcinoma cells were used to determine the effects of sulindac, and DFMO on cells containing an activated Ki-ras. Caco-2 cells are one of the few colon tumor lines which contain a normal Ki-ras gene (Delage et al., 1993; Trainer et al., 1988). These cells possess many of the characteristics of mature enterocytes, including spontaneous differentiation and hydrolase secretion (Rousset, 1986). Caco-2 cells were stably transfected with a plasmid directing the overexpression of an activated Ki-ras, and individual clones were subsequently isolated. Ten clonal populations were characterized by western blot employing an antibody that reacts with both normal and mutated Ki-ras proteins. (FIG. 1). Ki-ras protein was detectable in the parental cells, and was present in increased amounts in most of the transfectants. One clone, 13, had levels of Ki-ras below that of the parental Caco-2 cells, whereas clones 14, 22, 59, 80, 74, and 87 were intermediate expressors, and contained levels of Ki-ras similar to the uncloned Ki-ras transfected population (Caco-2/Ki-ras). Clones 60, 66, and 96 showed the highest level of expression. Clones 60 and 66 were utilized for further study.

Detailed Description Text (80):
Effect of sulindac sulfone and sulfide on growth of cells expressing an activated Ki-ras

Detailed Description Text (81):
Growth of parental Caco-2 cells as well as clones 60 and 66 were measured in the presence of vehicle, 120 .mu.M sulindac sulfide, or 600 .mu.M sulindac sulfone (FIG. 2). In the presence of vehicle only (2A), the expression of activated Ki-ras did not confer a growth advantage to either transfected cell line. The number of viable cells in all sulfide-treated cultures remained relatively unchanged throughout the 10-day growth period (2B), whereas treatment of cultures with the sulfone derivative resulted in a pronounced cell loss beginning 6 days after treatment (2C). Ki-ras activation did not confer a selective toxicity in the presence of either sulindac metabolite.

Detailed Description Text (82):
Effect of Ki-ras on apoptosis induced by sulindac sulfide or sulfone.

Detailed Description Text (83):
Sulindac has been shown to cause apoptosis in the AOM-treated rodent model. In this model, sulindac also reduces the number of tumors harboring an activated Ki-ras, which suggests that apoptosis may occur by a Ki-ras dependent mechanism. To test this hypothesis, Caco-2 parental cells and clones 60 and 66 were treated with vehicle (FIG. 3A), 120 .mu.M sulindac sulfide FIG. (3B), or 600 .mu.M sulindac sulfone FIG. (3C) and apoptosis of each culture was quantitated by light microscopy. The percentage of cells undergoing apoptosis in response to vehicle alone were minimal, with a peak apoptosis index of 4 percent FIG. (3A). Caco-2 parental cells exposed to sulfide displayed a gradual increase in apoptosis which peaked at 8 days in culture. Clone 60 cells, and to a lesser extent, clone 66, displayed an earlier onset of apoptosis. Clone 60 reached peak apoptosis levels at day 4 compared to day 8 of the parental cells, while clone 6 reached a peak level of apoptosis by day 6. In both the Caco-2 parental and clone 60 cells, induction of apoptosis by sulindac sulfone was more pronounced than with the sulfide FIG. (3C). Upon exposure to the sulfone, clone 60 cells reached a peak level of apoptosis by day 6, compared to day 8 in the parental cells. Ki-ras appears to accelerate the onset of apoptosis in response to both sulindac sulfone and sulfide.

Detailed Description Text (84):
Effect of Ki-ras activation on the survival of cells exposed to sulindac sulfide or sulfone

Detailed Description Text (85):
In an effort to better characterize the apoptosis response to the sulindac metabolites in cells with an activated Ki-ras, the colony-forming efficiencies of Caco-2 and clone 60 cells were measured after exposure to increasing concentrations

of sulindac sulfide or sulfone (FIG. 4). Cells were treated for 24 hr and then replated at diluted concentrations in fresh media. By this method, a 50% decrease in cell number occurred at approximately 225 .mu.M sulindac sulfide and 800 .mu.M sulindac sulfone. The expression of an activated Ki-ras did not affect the final measure of viability caused by either sulindac metabolite, as the transfected cells displayed the same toxicity as the parental cells. Activation of Ki-ras was unable to confer a selective cytotoxicity in the presence of either sulindac metabolite.

Detailed Description Text (86):
Effect of DFMO on the colony-forming efficiency of sulindac sulfone- and sulfide-treated Caco-2 cells

Detailed Description Text (87):
Caco-2 cells and Ki-ras transfectants exhibit a growth cessation in response to polyamide depletion, which is not accompanied by a loss of viability in short-term experiments. Polyamine depletion in these experiments appears to have no effect on sulindac-induced cytotoxicity. To further characterize the effects of polyamine depletion, both alone and in combination with sulindac, colony forming efficiencies of Caco-2 and Clone 60 cells were analyzed after exposure to 5 mM DFMO and increasing concentrations of sulindac sulfide or sulfone (FIG. 5). Exposure of the Ki-ras transfected clones to 5 mM DFMO had a much more pronounced effect on cell survival than that exhibited by the parental Caco-2 cells. Treatment of the transfected cells with 5 mM DFMO alone resulted in a 60% decrease in cell survival compared to untreated transfectants, whereas in the parental cells, DFMO treatment alone had little effect. For both Caco-2 and clone 60 cell lines, the combination of DFMO and increasing concentrations of either sulindac metabolite resulted in an even further decrease in cell survival, which suggests that DFMO did not inhibit sulindac-induced cytotoxicity.

Detailed Description Text (88):
When the survival of DFMO-treated clone 60 or parental Caco-2 cells was normalized to like DFMO-treated controls, the survival rates of the DFMO-treated cells mirrored those of the non-DFMO-treated cells in response to increasing concentrations of sulindac sulfide (FIG. 6). Clone 60, which expressed an activated Ki-ras, was slightly more resistant to the effects of sulindac sulfone than the parental cells, regardless of whether or not DFMO was present.

Detailed Description Text (89):
Effect of increasing concentrations of DFMO on survival of cells with an activated Ki-ras

Detailed Description Text (90):
The 60% decrease in survival of clone 60 clones treated with 5 mM DFMO suggests that polyamide was selectively toxic to cells expressing an activated Ki-ras. To further examine this response, Caco-2 cells and clones 60 and 66 were treated with increasing concentrations of DFMO for 24 hr and then replated for colony formation (FIG. 7). Caco-2 cells were refractory to DFMO treatment, and maintained nearly 100% survival at concentrations up to 5 mM DFMO. In contrast, both clone 60 and 66 exhibited increasing toxicity with increasing concentrations of DFMO, with 50% cell survival occurring at only 0.5 mM DFMO. At 5 mM DFMO, cell survival for both transfectants was decreased to 20-40% of controls. In these experiments, polyamine depletion was selectively toxic to cells with an activated Ki-ras at concentrations as low as 50 .mu.M.

Detailed Description Text (91):
The effect of DFMO on apoptosis induced by sulfide or sulfone in Caco-2 parental or Ki-ras transfected cells

Detailed Description Text (92):
Caco-2 cells or Caco-2 Ki-ras clone 60 were seeded in the presence or absence of 5 mM DFMO with or without 600 mM sulindac sulfone. Cells were grown over a period of 6 days. Cells were harvested at various days (0, 2, 4 and 6 days), spun onto slides and stained. Apoptosis was determined by light microscopy. Percentage of apoptotic cells was calculated as the number of apoptotic cells out of the total cells counted times 100. Results are shown in FIG. 8. Parental Caco-2 cells demonstrate only a

modest increase in apoptosis upon treatment with DFMO or DFMO and sulindac sulfone (FIG. 8A). This is in contrast to the results of treating Caco-2 Ki-ras clone 60 with DFMO or DFMO and sulindac sulfone (FIG. 8B). DFMO alone increased apoptosis from low background levels of 2 to 4% to a high of 15% on day 6, while the combination DFMO and sulindac sulfone increased apoptosis to 33% on day 6 in clone 60.

Detailed Description Text (94):
In the present study, the polyamine synthesis inhibitor .alpha.-difluoromethylomithine (DFMO) was selectively toxic to cells containing an activated Ki-ras. Ki-ras activation did not appear to render cells susceptible to cytotoxicity exerted by either metabolite of the NSAID sulindac. This suggests that the antitumor effects of DFMO and the NSAIDs seen in the AOM-rat model occur through different mechanisms.

Detailed Description Text (95):
The expression of an activated Ki-ras did not appear to change the growth rate of transfected cells, even in clones which expressed high levels of the protein nor did sulindac sulfide or sulfone exert a Ki-ras-specific effect on growth. This data is in contrast to work demonstrating a positive correlation between Ki-ras expression and growth rates in DLD-1 and HCT 116 human colon cell lines (Sharisawa et al., 1993).

Detailed Description Text (96):
A number of studies suggest that NSAID-mediated chemoprevention occurs through the induction of apoptosis (Bedi et al., 1995; Piazza et al., 1995; Boolbol et al., 1996; Samaha et al., 1997), however the role of Ki-ras activation in this process has not been extensively investigated. One group has found that Ki-ras activation in rat enterocytes confers resistance to apoptosis induced by sulindac sulfide although not with sulfone (Arber et al., 1997). In the present study, treatment of transfected Caco-2 cells with sulindac sulfide and sulfone resulted in a Ki-ras-dependent induction of apoptosis. Apoptosis induction by both sulfide and sulfone occurred several days earlier in Ki-ras transfected cells than in parental cells, although mammal levels of apoptosis in both transfectants and parental cells were similar. The efficacy of sulindac sulfone suggests the involvement of both prostaglandin-dependent and independent mechanisms.

Detailed Description Text (97):
The Ki-ras-dependent acceleration of apoptosis seen with sulindac treatment appeared to be inconsequential with respect to overall cytotoxicity. Survival studies with increasing concentrations of sulindac sulfide or sulfone did not reveal any differences in colony-forming efficiency between Ki-ras transfectants and parental Caco-2 cells, though cytotoxicity in both cell types was correlated with increased drug concentrations. This data suggests that although Ki-ras activation may render cells more susceptible to programmed cell death, the final level of cell survival influenced by sulindac metabolites occurred through a Ki-ras-independent mechanism.

Detailed Description Text (99):
Administration of both piroxicam and DFMO in the AOM-rat model is synergistically chemopreventive, decreasing both the number of existing tumors as well as the number of tumors with an activated Ki-ras (Ready et al., 1990). In the present study, treatment of Caco-2 cells with DFMO did not increase the toxicity of the sulindac metabolites in long-term survival studies. Thus, DFMO and sulindac may utilize different mechanisms to prevent tumorigenesis.

Detailed Description Text (100):
This possibility is further supported by the selective cytotoxicity of DFMO seen in cells with an activated Ki-ras. Treatment of both Ki-ras clone 60 and 66 with increasing concentrations of DFMO led to a dose-dependent decrease in colony-forming efficiency, whereas survival of parental cells was unaffected. Twenty-four hours of treatment with 5 mM DFMO was sufficient to reduce colony-forming efficiency of the Ki-ras transfectants by 40%, even though cells were subsequently incubated in normal media for an additional 24 hours prior to replating. Concentrations as low as 50 .mu.M DFMO were also moderately cytotoxic. In short-term growth studies, where cells were exposed to 5 mM DFMO continuously over a Sway time course, exclusion of trypan

blue dye in either the Ki-ras cells or parentals was unaffected. The cells appeared to be metabolically active during this time period, yet the colony-forming efficiency data indicate they were unable to proliferate. The cytotoxicity of DFMO on cells with an activated Ki-ras is consistent with other studies showing potent chemopreventive effects of DFMO on AOM-induced tumorigenesis as well as the development of tumors with an activated Ki-ras (Singh et al., 1994; Kulkarni et al., 1992).

Detailed Description Text (101):
The mechanism by which DFMO prevents Ki-ras-dependent tumorigenesis in the AOM-rat model is unknown. It has been proposed that sulindac and DFMO act through a common pathway involving the inhibition of prostaglandin synthesis (Ready et al., 1990), since ODC activity can be inhibited by agents which inhibit cyclooxygenases (Reddy et al., 1990; Reddy et al., 1988). The induction of apoptosis may occur through the inhibition of prostaglandin synthesis, or in the case of sulindac sulfone, a prostaglandin-independent mechanism that is also independent of Ki-ras activation. The activation of Ki-ras may lead to the downregulation of genes that are not necessary for growth, but are necessary for cell survival. This down regulation may not cause toxicity unless the cell undergoes the additional stress of polyamine depletion, in which case the genes normally expressed during conditions of stress are not available to protect the cell from a cytotoxic response.

Detailed Description Text (108):
Proliferating Cell Nuclear Antigen

Detailed Description Text (110):
Apoptosis Staining

Detailed Description Text (111):
Alterations in nuclear morphology (condensed chromatin, distinct apoptotic bodies) was the main endpoint used to score tissue in well oriented crypts and villi using standardized protocols to calculate the percent apoptotic cells per total counted.

Detailed Description Text (123):
(1) To measure the efficacy of DFMO plus Sulindac vs. placebo in modulating a panel of surrogate endpoint biomarkers (SEB) of particular relevance in colorectal neoplasia. Several measurements of quantitative histopathology and assessment of uninduced apoptosis, proliferative (Ki67) and preneoplastic (CEA, sialyl-TN, p53, bcl-2) features by immunoperoxidase will be done in biopsies of flat mucosa; polyamine and PGE.sub.2 levels will also be determined as estimate of biochemical effect by the two agents.

Detailed Description Text (155):
Chemoprevention studies of Ibuprofen, Piroxicam, and Sulindac against colon carcinogenesis have been reported in animal models (Moorghen et al., 1988; Pollard et al., 1989; Reddy et al., 1987). The major mechanisms by which this effect are mediated is not clearly defined but interactions with the cell cycle and apoptotic responses are likely (Pasricha et al., 1995; Piazza et al., 1995; Shiff et al., 1996; Shiff et al., 1995). The inventors have recently completed a phase Ia trial (one month) of Ibuprofen and even at the dose of 300 mg/day a significant decrease in PGE.sub.2 levels in flat mucosa was seen. A dose of 300 mg of Ibuprofen is very low (therapeutic doses range from 1200-3000 mg/day or more) and toxicity is unlikely to be seen, even over the long-term. However in animal chemoprevention models Ibuprofen is less effective than other NSAIDs. Although Piroxicam is the most effective chemoprevention agent in animal models (Pollard et al., 1989; Reddy et al., 1987), it demonstrated side effects in the recent IIb trial. A large meta-analysis of the side effects of the NSAIDs also indicates that piroxicam has more side effects than other NSAIDs (Lanza et al., 1995). Sulindac has been shown to produce regression of adenomas in FAP patients (Muscat et al., 1994). Although at least one study in sporadic adenomas has shown no such effect (Ladenheim et al., 1995), the inventors have recently completed a randomized phase IIb study that shows that Sulindac is safe and that this compound may cause polyp regression and inhibit the development of new polyps as well (DiSario et al., 1997). Although the study was relatively small, a dose of 150 mg per day vs. twice per day appeared to produce comparable results. Although arguments can be made pro and con for the choice of

NSAIDs, the assessment suggests that the efficacy of Sulindac in causing regression of human adenomas and its favorable safety/toxicity favors its use in combination trials compared to other NSAIDs. The inventors have therefore selected Sulindac at a dose of 150 mg per day as the NSAID to be used in the current example.

Detailed Description Text (158):
The effect of DFMO and Sulindac on modulating the relevant biochemical effect in the target organ of interest (flat colonic mucosa) is determined by serial measurements respectively of polyamines (putrescine levels and Spd/Spm ratio) and PGE.sub.2 levels.

Detailed Description Text (170):
measurement of uninduced apoptosis.

Detailed Description Text (186):
After morphological assessment on hematoxylin and eosin stains, sections are stained for markers for proliferation and pre-malignancy. the best of the former is MIB-47 1, which is reactive with a formalin-resistant epitope of KI-67. Expression of this marker shows a high correlation with S-phase fraction by flow cytometry (Keshgegian and Cnaan, 1995). Proliferation is determined from the percentage of 500 cells displaying nuclear immunoreactivity. Two useful pre-neoplastic markers are CEA and sialyl-Tn (Xu et al., 1989). Immunoreactivity is estimated by grading mucosal cells as negative, weakly positive, or strongly positive, or by optical density equantitation using the CAS system. The percentage of cells is determined for each staining category. Additional preneoplastic markers can be added to this panel as they become available (i.e., Bcl-2, NM (Liao et al., 1994), CMU 10 (Yang and Shamsuddin, 1995). Finally, p53 is assessed using the D07 antibody, which detects both native and mutant p53 (Voytesek et al., 1992). Although p53 mutation is considered a relatively late feature of colon carcinogenesis, a quantitative assessment of p53 staining nuclei (% p53 positive nuclei in 500 cells) may detect subtle early lesions in the mucosa. The main disadvantage of p53 assessment using immunoperoxidase is that if only a few cells are positive, whether they are mutated or wild-type p53 cannot be easily determined.

Detailed Description Text (188):
The paraffin embedded tissue is also be used for morphometry. Nuclei in mucosal crypts and adenomas can overlap substantially, which makes image analysis from cut sections inaccurate. Therefore, 50 .mu.m sections are micro-dissected to remove stroma and disaggregated using a procedure supplied by Becton Dicksinson. Briefly sections are deparaffinized, rehydrated, pepsin treated, cytocentrifuged and Feulgen stained. These preparations are used for both ploidy and analysis and nuclear measurements. The major disadvantage of this approach is that stromal cells and epithelial cells are not always readily distinguishable in these preparations. If necessary, sections are stained with anti-keratin antibodies by immunoperoxidase to confirm that the nuclei analyzed are epithelial in origin.

Detailed Description Text (189):
The methods for image analysis optimized for the CAS 200 Image Processing System (Becton Dickinson, San Jose, Calif.) are well characterized and have been described in the literature (Bacus and Bacus, 1994; Bacus and Grace, 1987). Briefly, approximately 200-500 Feulgen-stained nuclei displayed on the computer screen are automatically chosen by the computer. Operator intervention may be necessary to pick optimum nuclei. The computer calculates the optical density (OD) per nucleus, which is proportional to the DNA content. From this measure a histogram of the percentage of cells at each OD can be constructed. The peak indicates the ploidy, which is compared to a standard diploid cell population such as rat hepatocyte nuclei.

Detailed Description Text (190):
Using the cell Morphology Program of the CAS 200, additional measurements, such as nuclear area, shape and texture is obtained. Area is determined from the total number of pixels per nucleus and shape is computed from a combination of perimeter measurements and object size. The texture is determined by computing the standard deviation of OD over all the individual pixels in the nucleus. In addition, Markovian texture measurements can be calculated by comparing a pixel OD to the ODs of the nearest neighbors. Twenty-two Markovian texture calculations are available on

the software of the CAS 200 system (Bacus and Grace, 1987).

Detailed Description Text (191):
Determination of nucleolar size also takes advantage of the micrometer function of the CAS 200 system. In Feulgein-stained preparations, the nucleoli do not stain, but are surrounded by chromatin and appear as a hole. The radius of this circle can be determined and averaged over all the nuclei in the chosen sample. Similarly, the average number of nucleoli per cell can be determined by counting the total number of nucleoli in the nuclear sample and dividing by the total number of nuclei in the sample.

Detailed Description Text (200):
Using this information regarding sources of error, the inventors also measured ODC activity and polyamine contents in tissue samples obtained during colonscopy from 48 benign neoplastic polyps (20 tubular adenomas, 28 villous adenomas), 18 cancers (including 5 malignant polyps) and adjacent mucosa. ODC activity in polyp and cancer tissue specimens was higher than in adjacent mucosa in 75 and 83% of pairs, respectively. Similarly, putrescine, spermidine and spermine contents and polyamine content in colonic mucosa from 10 patients without a history of colorectal neoplasia were not different from adjacent mucosal values in the patients with neoplasia. From these measurements, the inventors concluded that ODC and polyamine contents are elevated in majority of colorectal neoplasia, but amounts in normal mucosa do not differentiate between patients with cancer, benign neoplastic polyps and normal subjects. Thus neither ODC activity nor polyamine contents of normal mucosa appear to be discriminatory markers of colorectal carcinogenesis. However, spd:spm rations, which show the least variability among measures of polyamine contents, were a good marker of the consequence of polyamine synthesis inhibition in chemoprevention trials. Details regarding the results summarized in this section have been published (Hixson et al., 1993; Hixson et al., 1994; Einspahr et al., 1995).

Detailed Description Text (203):
When the inventors started these studies, their hope was that the effects of polyamine syntheses inhibitors in easily accessible tissues, such as buccal mucosa in the oral cavity, would be representative of inhibitor effects in less accessible tissue of the gastrointestinal tract. To test this hypothesis, the inventors measured ODC activity and polyamine contents in buccal mucosa isolated from normal human volunteers. The inventors found that the majority of ODC activity in buccal mucosa was due to oral bacteria that was not separable from the human cells by extensive washing procedures. While DFMO reduced polyamine contents in colorectal tissue biopsies, buccal mucosal ODC and polyamine contents were unaffected. From these studies, the inventors concluded that the buccal mucosal was not a useful surrogate tissue in which to measure the effects of polyamine synthesis inhibitor on other GI tissues.

Detailed Description Text (205):
In the Barrett's esophagus group, patients were treated with DFMO 1.5 gm/m.sup.2 per day) for 12 weeks. Four sites (Barrett's lesion, adjacent normal squamous esophagus, gastric tissue and small bowel) were biopsied in each patient before during and after DFMO treatment in order to assess the effects of this drug on tissue polyamine levels. ODC activities and polyamine contents varied in each site analyzed. The rank orders were Barrett's>small bowel.about.normal esophagus>gastric tissue for ODC activities, and small bowel.gtoreq.Barrett's.about.normal esophagus>gastric tissue for putrescine contents. Spermidine, but not spermine, contents in Barrett's lesions and normal squamous esophageal tissue were suppressed by systemic DFMO treatment and recovered to untreated control values when DFMO therapy was discontinued. Systemic DFMO treatment did not affect the levels of either of these two amines in gastric tissue and small bowel. Since DFMO can suppress polyamine contents in several human gastrointestinal tissues, including Barrett's mucosa, the inventors concluded that DFMO would be an effective agent to test the hypothesis that depletion of spermidine contents may prevent the development of adenocarcinoma of the specific patient group.

Detailed Description Text (206):
In the group, 111 patients, who had undergone colonscopy for surgical removal of an adenomatous colon polyp greater than 5 mm within 5 years prior to enter the study,

were treated with DFMO for 4 wk to determine the lowest does of this agent which would suppress colorectal tissue polyamine contents. A dose de-escalation trial design was employed, in which groups of patients (12-20 patients per group) were treated with single daily doses of DFMO ranging from 3.0 to 0.1 gm/m.sup.2. Prior to initiation of DFMO treatment and at the end of treatment, six colorectal biopsy specimens were collected from patient along with serum samples. All biopsies were performed between 9:00 a.m. and noon to avoid possible effects of diurnal variation in laboratory endpoints. Samples for analysis of plasma DFMO levels were also collected during this time period of the day after the last day of drug administration. DFMO caused a decrease in both putrescine content and the ration of spd:smp for all dose groups down to 0.25 gm/m.sup.2. Both putrescine content and the spd:smp ratio, and changes in these parameters as a function of DFMO treatment, decreased as function of donor age. None of the 30 patients receiving either 0.25 or 0.5 gm/m.sup.2 experienced any clinical ototoxicity in this trial. The inventors concluded that DFMO was both safe and effective in reducing colorectal mucosal polyamine contents when administered daily orally to patients at doses as low as 0.25 gm/m.sup.2 for 28 days. No ototoxicity was observed at doses up to twice this amount. Details of this work have been published (Meyskens et al., 1994; Boyle et al., 1992; Gerner et al., 1994; Meyskens et al., 1992; Meyskens et al., 1995).

Detailed Description Text (207):
Measurement of cell death, including apoptosis.

Detailed Description Text (208):
Apoptosis and necrosis are two distinct modes of cell death (Walker et al., 1988; Searle et al., 1982; Wyllie, 1981; Kerr et al., 1972; Kerr et al., 1987). Apoptosis is a controlled mode of cell death in which the dying cell takes an active role in its own demise. Classical necrosis, however, is a traumatic or accidental mode of cell death resulting, for example, from extremes in environmental conditions. John Kerr first recognized apoptosis as a distinct mode of cell death in 1972, and described this process at the ultrastructural level (Kerr et al., 1972). The sub-cellular features that characterize apoptotic cells include a condensation and margination of the chromatin, increased electron density, cytoplasmic vacuolization, fragmentation of the nucleus into membrane-bound bodies, and apoptotic body formation (Kerr et al., 1972; Payne et al., 1992). Time-lapse cinematographic studies indicate the dramatic surface changes that accompany apoptosis ("cell boiling" and shrinkage) and the explosive nature (cell swelling and membrane rupture) of a cell undergoing necrosis (Matter, 1979; Russell et al., 1972; Sanderson, 1981).

Detailed Description Text (209):
Since these early ultrastructural studies, many light (Payne et al., 1995) and biochemical assays have been utilized to identify apoptotic cells to replace the time-consuming ultrastructural evaluations. The inventors are using bright field microscopic methods as the main technique for assessing apoptosis in the proposed studies.

Detailed Description Text (210):
Apoptosis in uninduced tissue is measured in apparently normal colonic mucosa and in colon polyps. Preliminary studies in the inventors' group to date, in both experimental animal models (rodents) and humans, indicate that the baseline values of apoptosis in apparently normal colonic mucosa are heterogeneous (generally apoptotic cells are found at the top of the crypt, less frequently at the base) and low (less than 4%).

Detailed Description Text (211):
The tissue is fixed in formalin and three micron sections are then prepared using glass knives, stained with Methylene Blue/Azure II/Basic Fuchsin (a polychrome stain) and the number of cells with "darkly-stained" (apoptotic) and "lightly-stained" (non-apoptotic) nuclei is determined by light microscopy.

Detailed Description Text (212):
All cells in the crypts are stored in this assay. The criteria used to identify apoptotic cells are based on the increased staining intensity of the nuclei and is determined from at least 10 different crypts per biopsy. A minimum of 200 cells is

scored per biopsy. The identification of apoptotic cells by light microscopy has been confirmed by electron microscopy (Samaha et al., 1995; Samaha et al., 1995). Dark nuclei only indicated the early stage of apoptosis which is accompanied by mirgination and condensation of chromatin.

Detailed Description Text (213):
All apoptosis data is entered into a computer file, providing spatial information relating apoptotic cells to position in colonic crypts. This method of data analysis allows the inventors to compare patterns of basal levels of apoptosis as a function of their spatial position in the crypt. This is important, as recent studies have been indicated that other colon cancer-related gene defects, such as mutations in the adnenomatous polyposis coli (APC) gene, cause unique spatial changes in apoptosis in the apparently normal colonic mucosa (Strater et al., 1995).

Detailed Description Text (226):
Alberts, Hixson, Ahnen, Bogert, Einspahr, Paranak, Brendel, Gross, Pamukcu, Burt, "Do NSAIDs exert their colon cancer chemoprevention activities through the inhibition of mucosal prostaglandin synthetase?" J. Cell. Biochem. Supp., (22):18-23, 1995.

Detailed Description Text (227):
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Detailed Description Text (229):
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Detailed Description Text (232):
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Detailed Description Text (270):
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Detailed Description Text (281):
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Detailed Description Text (282):
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Detailed Description Text (283):
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Detailed Description Text (287):
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Detailed Description Text (288):
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Detailed Description Text (297):
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Detailed Description Text (301):
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Detailed Description Text (312):
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Detailed Description Text (320):
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Alberts et al., "Do NSAIDs exert their colon cancer chemoprevention activities through the inhibition of mucosal prostaglandin synthetase?" J. Cell. Biochem. Supp., (22):18-23, 1995.

Other Reference Publication (12):
Meyskens and Gerner, "Development of difluoromethylornithine as a chemoprevention agent for the management of colon cancer," J. Cell. Biochem., 22:126-131, 1995.

Other Reference Publication (14):
Piazza et al., "Antineoplastic drugs sulindac sulfide and sulfone inhibit cell growth by inducing apoptosis," Cancer Res., (55):311 3116, 1995.

Other Reference Publication (15):
Piazza et al., "Apoptosis primarily accounts for the growth-inhibitory properties of sulindac metabolites and involves a mechanism that is independent of cyclooxygenase inhibition, cell cycle arrest, and p53 induction," Cancer Res., (57):2452-2459, 1997.

Other Reference Publication (23):
Kelloff et al., "New agents for cancer chemoprevention," J. Cell. Biochem., 265:1-28, 1996.

Other Reference Publication (26):
Arber et al., "A K-ras oncogene increases resistance to sulindac-induced apoptosis in rat enterocytes," Gastroenterology, (113):1892-1990, 1997.

Other Reference Publication (28):
Pasricha et al., "The effects of sulindac on colorectal proliferation and apoptosis in familial adenomatous polyposis," Gastroenterology, 109:994-998, 1995.

Other Reference Publication (32):
Kelloff et al., "New agents for chemoprevention," J. of Cell. Biochemistry, 63:1-28, 1996.

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             39  EIF5A
           3639  EUCARYOTIC
          48696  EUKARYOTIC
         154311  INITIATION
        1185190  FACTOR
          10337  5A
      S1    245  EIF5A OR ((EUCARYOTIC OR EUKARYOTIC) AND INITIATION AND
                 FACTOR AND 5A)
? s s1 and apoptosis?
            245  S1
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      S2     16  S1 AND APOPTOSIS?
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             84  S4
         126397  APOPTOSIS?
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 5/3,AB/1     (Item 1 from file: 5)
DIALOG(R)File   5:Biosis Previews(R)
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13526822   BIOSIS NO.: 200200155643
Effects of N1-guanyl-1,7-diaminoheptane, an inhibitor of
  deoxyhypusine synthase, on endothelial cell growth,
  differentiation and apoptosis.
AUTHOR: Joe Young Ae(a); Lee Yoon; Kim Hyun-Kyung; Kim You Young; Park
  Myung Hee
AUTHOR ADDRESS: (a)Cancer Research Institute, The Catholic University of
  Korea, 505 Banpo-dong, Seocho-ku, Seoul, 137-701**South Korea
JOURNAL: Molecular Biology of the Cell  11 (Supplement):p455a Dec., 2000
MEDIUM: print
CONFERENCE/MEETING: 40th American Society for Cell Biology Annual Meeting
San Francisco, CA, USA  December 09-13, 2000
ISSN: 1059-1524
RECORD TYPE: Citation
LANGUAGE: English
2000
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...completed examining records
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? t s6/3,ab/all

 6/3,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12760145   21597923   PMID: 11762979
  "Tissue" transglutaminase expression in HIV-infected cells: an enzyme
with an antiviral effect?
  Amendola A; Rodolfo C; Di Caro A; Ciccosanti F; Falasca L; Piacentini M
  Laboratory of Virology, Lazzaro Spallanzani-IRCCS, Rome, Italy.
  Annals of the New York Academy of Sciences (United States)  Nov 2001,
946 p108-20,  ISSN 0077-8923  Journal Code: 7506858
  Document type: Journal Article; Review; Review, Tutorial
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Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The cytopathic effect of HIV has been shown to be associated with the induction of **apoptosis** and the inhibition of proliferation of T cells. However, the cellular and molecular mechanisms at the basis of the dramatic immune cell loss caused by HIV in patients suffering from acquired immunodeficient syndrome (AIDS), are not yet fully established. We demonstrated that "tissue" transglutaminase (tTG) gene expression is induced in the immune system of seropositive individuals (peripheral blood mononuclear cells and lymph nodes). tTG is a multifunctional protein involved in a variety of fundamentally important cellular functions, in addition to cell death by **apoptosis**. The presence of high tTG levels in immune-competent cells of HIV+ persons might exert an important role in HIV-infection by influencing viral production. We propose that, in addition to its multiple functions, tTG might interfere with HIV replication by altering the viral mRNA trafficking between the nucleus and the cytoplasm. This effect might be due to its specific interaction with **eIF5A**, a cellular partner of HIV Rev protein, which is essential for HIV replication in immune-competent cells. Given the presence of high tTG levels in HIV+ individuals, it would be of interest to pursue the potential role of this multifunctional protein in the development of strategies aimed at the pharmacologic regulation of HIV production.

6/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11197967 21229963 PMID: 11332455

The role of **eukaryotic initiation factor 5A** in the control of cell proliferation and **apoptosis**.

Caraglia M; Marra M; Giuberti G; D'Alessandro A M; Budillon A; del Prete S; Lentini A; Beninati S; Abbruzzese A

Dipartimento di Biochimica e Biofisica, Seconda Universita di Napoli, Italy.

Amino acids (Austria) 2001, 20 (2) p91-104, ISSN 0939-4451
Journal Code: 9200312

Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

In the past years, the attention of scientists has mainly focused on the study of the genetic information and alterations that regulate **eukaryotic** cell proliferation and that lead to neoplastic transformation. An increasing series of data are emerging about the involvement of the **initiation** phase of translational processes in the control of cell proliferation. In this paper we review the novel insights on the biochemical and molecular events leading to the **initiation** and its involvement in cell proliferation and tumourigenesis. We describe the structure, regulation and proposed functions of the **eukaryotic initiation factor 5A** (eIF-**5A**) focusing the attention on its involvement in the regulation of **apoptosis** and cell proliferation. Moreover, we describe the modulation of its activity (through the reduction of hypusine synthesis) in **apoptosis** induced either by tissue transglutaminase or interferon a. Finally, we propose eIF-**5A** as an additional target of anti-cancer strategies.

6/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10785648 20325068 PMID: 10866791

Modulation of molecular mechanisms involved in protein synthesis machinery as a new tool for the control of cell proliferation.

Caraglia M; Budillon A; Vitale G; Lupoli G; Tagliaferri P; Abbruzzese A
Dipartimento di Biochimica e Biofisica, Seconda Universita di Napoli, Italy.
European journal of biochemistry / FEBS (GERMANY) Jul 2000, 267 (13) p3919-36, ISSN 0014-2956 Journal Code: 0107600
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

In the past years, the attention of scientists has focused mainly on the study of the genetic information and alterations that regulate **eukaryotic** cell proliferation and that lead to neoplastic transformation. All therapeutic strategies against cancer are, to date, directed at DNA either with cytotoxic drugs or gene therapy. Little or no interest has been aroused by protein synthesis mechanisms. However, an increasing body of data is emerging about the involvement of translational processes and factors in control of cell proliferation, indicating that protein synthesis can be an additional target for anticancer strategies. In this paper we review the novel insights on the biochemical and molecular events leading to protein biosynthesis and we describe their involvement in cell proliferation and tumorigenesis. A possible mechanistic explanation is given by the interactions that occur between protein synthesis machinery and the proliferative signal transduction pathways and that are therefore suitable targets for indirect modulation of protein synthesis. We briefly describe the molecular tools used to block protein synthesis and the attempts made at increasing their efficacy. Finally, we propose a new multimodal strategy against cancer based on the simultaneous intervention on protein synthesis and signal transduction.

6/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10664048 20200865 PMID: 10736626
Post-translational modifications of **eukaryotic initiation factor-5A** (eIF-**5A** ) as a new target for anti-cancer therapy.
Caraglia M; Tagliaferri P; Budillon A; Abbruzzese A
Department of Biochemistry and Biophysics F. Cedrangolo, Second University of Naples, Italy.
Advances in experimental medicine and biology (UNITED STATES) 1999, 472 p187-98, ISSN 0065-2598 Journal Code: 0121103
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

**Eukaryotic** translation **initiation factor 5A** (eIF-**5A** ) is the only cell protein that contains the unusual basic amino acid hypusine [N epsilon-(4-amino-2-hydroxybutyl)lysine]. Hypusine is formed by the transfer of the butylamine portion from spermidine to the epsilon-amino group of a specific lysine residue of eIF-**5A** precursor and the subsequent hydroxylation at carbon 2 of the incoming 4-aminobutyl moiety. Agents that reduce cell hypusine levels inhibit the growth of mammalian cells. These observations suggest that hypusine is crucial for proliferation and transformation of **eukaryotic** cells. Here we have studied whether the inhibition of hypusine synthesis can potentiate the anti-cancer activity of the anti-tumour agents interferon-alpha (IFN alpha) and cytosine arabinoside (ara-C). We have found that IFN alpha increased epidermal growth **factor** receptor (EGF-R) expression, but reduced S phase and proliferative marker expression in human epidermoid KB cells and that this effect was antagonised by epidermal growth **factor** (EGF). Growth inhibition induced by IFN alpha was paralleled by decreased hypusine synthesis and, when EGF counteracted anti-proliferative effects, a reconstitution of hypusine levels was recorded. We also studied the effects

of IFN alpha on the cytotoxicity of the recombinant toxin TP40 which inhibits elongation **factor** 2, another step of protein synthesis, through EGF-R binding and internalisation; IFN alpha induced an about 27-fold increase of TP40 cytotoxicity in KB cells. Ara-C, another antineoplastic agent commonly used in haematologic malignancies, induced both **apoptosis** and iron depletion in human acute myeloid leukaemic cells. The combination of ara-C and of the iron chelator desferioxamine, a strong inhibitor of hypusine synthesis, had a synergistic activity on **apoptosis** in these cells. The data strongly suggest that the post-translational modifications of eIF-**5A** could be a suitable target for the potentiation of the activity of anti-cancer agents.

6/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10663528 20202622 PMID: 10737792
Expression analysis with oligonucleotide microarrays reveals that MYC regulates genes involved in growth, cell cycle, signaling, and adhesion.
Coller H A; Grandori C; Tamayo P; Colbert T; Lander E S; Eisenman R N; Golub T R
Center for Genome Research, Whitehead Institute for Biomedical Research, Cambridge, MA 02139, USA. hcoller@fhcrc.org
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Mar 28 2000, 97 (7) p3260-5, ISSN 0027-8424 Journal Code: 7505876
Contract/Grant No.: CA20525; CA; NCI; CA75125; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
MYC affects normal and neoplastic cell proliferation by altering gene expression, but the precise pathways remain unclear. We used oligonucleotide microarray analysis of 6,416 genes and expressed sequence tags to determine changes in gene expression caused by activation of c-MYC in primary human fibroblasts. In these experiments, 27 genes were consistently induced, and 9 genes were repressed. The identity of the genes revealed that MYC may affect many aspects of cell physiology altered in transformed cells: cell growth, cell cycle, adhesion, and cytoskeletal organization. Identified targets possibly linked to MYC's effects on cell growth include the nucleolar proteins nucleolin and fibrillarin, as well as the **eukaryotic initiation factor 5A**. Among the cell cycle genes identified as targets, the G1 cyclin D2 and the cyclin-dependent kinase binding protein CksHs2 were induced whereas the cyclin-dependent kinase inhibitor p21(Cip1) was repressed. A role for MYC in regulating cell adhesion and structure is suggested by repression of genes encoding the extracellular matrix proteins fibronectin and collagen, and the cytoskeletal protein tropomyosin. A possible mechanism for MYC-mediated **apoptosis** was revealed by identification of the tumor necrosis **factor** receptor associated protein TRAP1 as a MYC target. Finally, two immunophilins, peptidyl-prolyl cis-trans isomerase F and FKBP52, the latter of which plays a role in cell division in Arabidopsis, were up-regulated by MYC. We also explored pattern-matching methods as an alternative approach for identifying MYC target genes. The genes that displayed an expression profile most similar to endogenous Myc in microarray-based expression profiling of myeloid differentiation models were highly enriched for MYC target genes.

6/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09944365 98378101 PMID: 9714299
Antiretroviral effects of deoxyhypusyl hydroxylase inhibitors: a

hypusine-dependent host cell mechanism for replication of human immunodeficiency virus type 1 (HIV-1).
Andrus L; Szabo P; Grady R W; Hanauske A R; Huima-Byron T; Slowinska B; Zagulska S; Hanauske-Abel H M
The New York Blood Center, NY, USA.
Biochemical pharmacology (ENGLAND) Jun 1 1998, 55 (11) p1807-18, ISSN 0006-2952 Journal Code: 0101032
Contract/Grant No.: AI34773; AI; NIAID; P01AG00541; AG; NIA; R37AG08707; AG; NIA; +
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The HIV-1 protein Rev, critical for translation of incompletely spliced retroviral mRNAs encoding capsid elements, requires a host cell protein termed "**eukaryotic initiation factor 5A**" (eIF-**5A** ). This is the only protein containing hypusine, a lysine-derived hydroxylated residue that determines its proposed bioactivity, the translation of a subset of cellular mRNAs controlling G1-to-S transit of the cell cycle. We postulated that inhibiting the hypusine-forming deoxyhypusyl hydroxylase (DOHH) should, by depleting **eukaryotic initiation factor 5A** , compromise Rev function and thus reduce HIV-1 multiplication. We now report that the alpha-hydroxypyridones, specifically mimosine, a natural product, and deferiprone, an experimental drug, inhibited deoxyhypusyl hydroxylase in T-lymphocytic and promonocytic cell lines and, in a concentration-dependent manner, suppressed replication of HIV-1. However, the alpha-hydroxypyridones did not affect the formation of unspliced or multiply spliced HIV-1 transcripts. Rather, these agents caused Rev-dependent incompletely spliced HIV-1 mRNA such as gag, but not cellular "housekeeping" mRNAs, to disappear from polysomes. Consequently, alpha-hydroxypyridone-mediated depletion of eIF-**5A** decreased biosynthesis of structural HIV-1 protein encoded by gag, measured as p24, whereas the induced formation of cellular protein like tumor necrosis **factor** alpha remained unaffected. By interfering with the translation of incompletely spliced retroviral mRNAs, these compounds restrict HIV-1 to the early, nongenerative phase of its reproductive cycle. In the inducibly HIV-1 expressing T-cell line ACH-2, the deoxyhypusyl hydroxylase inhibitors triggered extensive **apoptosis** , particularly of cells that actively produce HIV-1. Selective suppression of retroviral protein biosynthesis and preferential **apoptosis** of retrovirally infected cells by alpha-hydroxypyridones point to a novel mode of antiretroviral action.

6/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09689842 98113151 PMID: 9442029
Identification of the **eukaryotic initiation factor 5A** as a retinoic acid-stimulated cellular binding partner for tissue transglutaminase II.
Singh U S; Li Q; Cerione R
Department of Pharmacology, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853, USA.
Journal of biological chemistry (UNITED STATES) Jan 23 1998, 273 (4) p1946-50, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: EY06429; EY; NEI; GM40654; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
GTP-binding protein/transglutaminases (tissue transglutaminases or TGases) have been implicated in a variety of cellular processes including retinoic acid (RA)-induced **apoptosis**. Recently, we have shown that RA activates TGases as reflected by stimulated GTP binding, increased membrane

association, and stimulated phosphoinositide lipid turnover. This prompted us to search for cellular proteins that bind TGases in a RA-stimulated manner. In this report, we show that the **eukaryotic initiation factor** (eIF-**5A** ), a protein that is essential for cell viability, perhaps through effects on protein synthesis and/or RNA export, associates with the TGase in vivo. The interaction between eIF-**5A** and TGase is specific for the GDP-bound form of the TGase and is not detected when the TGase is pre-loaded with GTP gamma S. The TGase-eIF-**5A** interaction also is promoted by Ca2+, Mg2+, and RA treatment of HeLa cells. In the presence of retinoic acid, millimolar levels of Ca2+ are no longer required for the TGase-eIF-**5A** interaction. Nocodazole treatment, which blocks the cell cycle at mitosis (M phase), strongly inhibits the interaction between eIF-**5A** and cytosolic TGase. The interaction between TGase and eIF-**5A** and its sensitivity to the nucleotide-occupied state of the TGase provides a potentially interesting connection between RA signaling and protein synthesis and/or RNA trafficking activities.

6/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09637936 98060742 PMID: 9396730
Excess putrescine accumulation inhibits the formation of modified **eukaryotic initiation factor 5A** (eIF-**5A**) and induces **apoptosis**.
Tome M E; Fiser S M; Payne C M; Gerner E W
Department of Radiation Oncology, Arizona Health Sciences Center, University of Arizona, Tucson, AZ 85724, USA.
Biochemical journal (ENGLAND) Dec 15 1997, 328 ( Pt 3) p847-54, ISSN 0264-6021 Journal Code: 2984726R
Contract/Grant No.: CA-23074; CA; NCI; CA-30052; CA; NCI; CA-72008; CA; NCI; +
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
DH23A cells, an alpha-difluoromethylornithine-resistant variant of the parental hepatoma tissue culture cells, express high levels of stable ornithine decarboxylase. Aberrantly high expression of ornithine decarboxylase results in a large accumulation of endogenous putrescine and increased **apoptosis** in DH23A cells when alpha-difluoromethylornithine is removed from the culture. Treatment of DH23A cells with exogenous putrescine in the presence of alpha-difluoromethylornithine mimics the effect of drug removal, suggesting that putrescine is a causative agent or trigger of **apoptosis**. Accumulation of excess intracellular putrescine inhibits the formation of hypusine in vivo, a reaction that proceeds by the transfer of the butylamine moiety of spermidine to a lysine residue in **eukaryotic initiation factor 5A** (eIF-**5A**). Treatment of DH23A cells with diaminoheptane, a competitive inhibitor of the post-translational modification of eIF-**5A** , causes both the suppression of eIF-**5A** modification in vivo and induction of **apoptosis** . These data support the hypothesis that rapid degradation of ornithine decarboxylase is a protective mechanism to avoid cell toxicity from putrescine accumulation. Further, these data suggest that suppression of modified eIF-**5A** formation is one mechanism by which cells may be induced to undergo **apoptosis**.

6/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09523055 97430999 PMID: 9285098
Cellular **eukaryotic initiation factor 5A** content

as a mediator of polyamine effects on growth and **apoptosis**.
Tome M E; Gerner E W
Department of Biochemistry, University of Arizona, Tucson 85724, USA.
Biological signals (SWITZERLAND) May-Jun 1997, 6 (3) p150-6, ISSN 1016-0922 Journal Code: 9210083
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The polyamines are essential for **eukaryotic** cell growth. One of the most critical effects of polyamines on cell growth is the availability of spermidine for the post-translational modification of eIF-**5A**. Because hypusine-containing eIF-**5A** is necessary for cell proliferation, depletion of cellular polyamines suppresses growth by depleting cellular modified eIF-**5A** content. Excess putrescine accumulations in DH23A/b cells induces **apoptosis** and suppresses the formation of hypusine-containing eIF-**5A** . Treatment of DH23A/b cells with diaminoheptane also suppresses modified eIF-**5A** formation and induces **apoptosis**. These data suggest that suppression of modified eIF-**5A** formation may play a role in putrescine-induced **apoptosis** as well.

6/3,AB/10 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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13526822 BIOSIS NO.: 200200155643
Effects of N1-guanyl-1,7-diaminoheptane, an inhibitor of deoxyhypusine synthase, on endothelial cell growth, differentiation and **apoptosis**
.
AUTHOR: Joe Young Ae(a); Lee Yoon; Kim Hyun-Kyung; Kim You Young; Park Myung Hee
AUTHOR ADDRESS: (a)Cancer Research Institute, The Catholic University of Korea, 505 Banpo-dong, Seocho-ku, Seoul, 137-701**South Korea
JOURNAL: Molecular Biology of the Cell 11 (Supplement):p455a Dec., 2000
MEDIUM: print
CONFERENCE/MEETING: 40th American Society for Cell Biology Annual Meeting San Francisco, CA, USA December 09-13, 2000
ISSN: 1059-1524
RECORD TYPE: Citation
LANGUAGE: English
2000
? ds

| Set | Items | Description |
|---|---|---|
| S1 | 245 | EIF5A OR ((EUCARYOTIC OR EUKARYOTIC) AND INITIATION AND FACTOR AND 5A) |
| S2 | 16 | S1 AND APOPTOSIS? |
| S3 | 10 | S1 AND DHS |
| S4 | 84 | S1 AND DEOXYHYPUSINE AND SYNTHASE? |
| S5 | 1 | S4 AND APOPTOSIS? |
| S6 | 10 | RD S2 (unique items) |

? rd s3
...completed examining records
S7 5 RD S3 (unique items)
? t s7/3,ab/all

7/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11229092 21264522 PMID: 11278418
Isolation and characterization of senescence-induced cDNAs encoding deoxyhypusine synthase and **eucaryotic** translation **initiation**

**factor 5A** from tomato.
Wang T W; Lu L; Wang D; Thompson J E
Department of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada.
Journal of biological chemistry (United States) May 18 2001, 276 (20) p17541-9, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Full-length cDNA clones encoding deoxyhypusine synthase (**DHS**) and **eucaryotic initiation factor 5A** (eIF-**5A**) have been isolated from a cDNA expression library prepared from tomato leaves (Lycopersicon esculentum, cv. Match) exposed to environmental stress. **DHS** mediates the first of two enzymatic reactions that activate eIF-**5A** by converting a conserved lysine to the unusual amino acid, deoxyhypusine. Recombinant protein obtained by expressing tomato **DHS** cDNA in Escherichia coli proved capable of carrying out the deoxyhypusine synthase reaction in vitro in the presence of eIF-**5A**. Of particular interest is the finding that **DHS** mRNA and eIF-**5A** mRNA show a parallel increase in abundance in senescing tomato flowers, senescing tomato fruit, and environmentally stressed tomato leaves exhibiting programmed cell death. Western blot analyses indicated that **DHS** protein also increases at the onset of senescence. It is apparent from previous studies with yeast and mammalian cells that hypusine-modified eIF-**5A** facilitates the translation of a subset of mRNAs mediating cell division. The present study provides evidence for senescence-induced **DHS** and eIF-**5A** in tomato tissues that may facilitate the translation of mRNA species required for programmed cell death.

7/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11051977 21037603 PMID: 11197320
Phylogenetic origin of a secondary pathway: the case of pyrrolizidine alkaloids.
Ober D; Hartmann T
Institut fur Pharmazeutische Biologie, Technische Universitat Braunschweig, Germany.
Plant molecular biology (Netherlands) Nov 2000, 44 (4) p445-50, ISSN 0167-4412 Journal Code: 9106343
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Recent studies have revealed high sequence similarity between homospermidine synthase (HSS), the first pathway-specific enzyme in the biosynthesis of pyrrolizidine alkaloids, a class of sporadically occurring plant defence compounds, and deoxyhypusine synthase (**DHS** ), a ubiquitous enzyme involved in the post-translational activation of the **eukaryotic initiation factor 5A** (**eIF5A**). The recruitment of **DHS** during the evolution of the alkaloid pathway is discussed and interpreted as evolution by change of function.

7/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10546636 20079558 PMID: 10611289
Homospermidine synthase, the first pathway-specific enzyme of pyrrolizidine alkaloid biosynthesis, evolved from deoxyhypusine synthase.
Ober D; Hartmann T
Institut fur Pharmazeutische Biologie der Technischen Universitat

Braunschweig, Mendelssohnstrasse 1, D-38106 Braunschweig, Germany.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Dec 21 1999, 96 (26) p14777-82, ISSN 0027-8424 Journal Code: 7505876
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Pyrrolizidine alkaloids are preformed plant defense compounds with sporadic phylogenetic distribution. They are thought to have evolved in response to the selective pressure of herbivory. The first pathway-specific intermediate of these alkaloids is the rare polyamine homospermidine, which is synthesized by homospermidine synthase (HSS). The HSS gene from Senecio vernalis was cloned and shown to be derived from the deoxyhypusine synthase (**DHS** ) gene, which is highly conserved among all eukaryotes and archaebacteria. **DHS** catalyzes the first step in the activation of translation **initiation factor 5A** (**eIF5A**), which is essential for **eukaryotic** cell proliferation and which acts as a cofactor of the HIV-1 Rev regulatory protein. Sequence comparison provides direct evidence for the evolutionary recruitment of an essential gene of primary metabolism (**DHS**) for the origin of the committing step (HSS) in the biosynthesis of pyrrolizidine alkaloids.

7/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09725517 98154315 PMID: 9493264
Crystal structure of the NAD complex of human deoxyhypusine synthase: an enzyme with a ball-and-chain mechanism for blocking the active site.
Liao D I; Wolff E C; Park M H; Davies D R
Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, MD 20892-0560, USA.
Structure (London, England) (ENGLAND) Jan 15 1998, 6 (1) p23-32, ISSN 0969-2126 Journal Code: 9418985
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND: **Eukaryotic initiation factor 5A** (eIF-**5A** ) contains an unusual amino acid, hypusine [N epsilon-(4-aminobutyl-2-hydroxy)lysine]. The first step in the post-translational formation of hypusine is catalysed by the enzyme deoxyhypusine synthase (**DHS**). The modified version of eIF-**5A**, and **DHS**, are required for **eukaryotic** cell proliferation. Knowledge of the three-dimensional structure of this key enzyme should permit the design of specific inhibitors that may be useful as anti-proliferative agents. RESULTS: The crystal structure of human **DHS** with bound NAD cofactor has been determined and refined at 2.2 A resolution. The enzyme is a tetramer of four identical subunits arranged with 222 symmetry; each subunit contains a nucleotide-binding (or Rossmann) fold. The tetramer comprises two tightly associated dimers and contains four active sites, two in each dimer interface. The catalytic portion of each active site is located in one subunit while the NAD-binding site is located in the other. The entrance to the active-site cavity is blocked by a two-turn alpha helix, part of a third subunit, to which it is joined by an extended loop. CONCLUSIONS: The active site of **DHS** is a cavity buried below the surface of the enzyme at the interface between two subunits. In the conformation observed here, the substrate-binding site is inaccessible and we propose that the reaction steps carried out by the enzyme must be accompanied by significant conformational changes, the least of which would be the displacement of the two-turn alpha helix.

7/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09463365 97386335 PMID: 9244184
Effects of N1-guanyl-1,7-diaminoheptane, an inhibitor of deoxyhypusine synthase, on the growth of tumorigenic cell lines in culture.
Shi X P; Yin K C; Ahern J; Davis L J; Stern A M; Waxman L
Department of Biological Chemistry, Merck Research Laboratories, West Point, Pennsylvania 19486, USA. Shi@merck.com
Biochimica et biophysica acta (NETHERLANDS) Jan 10 1996, 1310 (1) p119-26, ISSN 0006-3002 Journal Code: 0217513
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
N1-guanyl-1,7-diaminoheptane (GC7) is a potent inhibitor of deoxyhypusine synthase (**DHS** ), the enzyme that catalyzes the first step in the hypusination of **eukaryotic** translation **initiation factor 5A** (eIF-**5A**). Since eIF-**5A** is the only known cellular substrate for **DHS** and GC7 has been reported to block the proliferation of CHO cells, it has been suggested that **DHS** may be a novel target for anti-cancer therapy. In the present study we investigated the antiproliferative effect of GC7 on several tumorigenic cell lines under various growth conditions. We found that this compound inhibits the proliferation of H9 cells in suspension culture and the growth of HeLa cells and v-src-transformed NIH3T3 cells under both anchorage-dependent and anchorage-independent conditions. Moreover, studies with NIH3T3 cells and v-src-transformed NIH3T3 cells show that GC7 inhibits the growth of both cell lines in monolayer culture with similar potency and could not reverse the transformed phenotype. In addition, the v-src-transformed cells grown under both anchorage-dependent and anchorage-independent conditions showed similar sensitivity toward GC7. These data indicate that GC7 acts as a general antiproliferative agent and does not appear to preferentially target tumorigenic cell types. Cell cycle analysis show that GC7 reduces the CHO-K1 cell population in the G1-phase of the cell cycle by 42% and increases the number of cells in the S-phase by 44%. This cell cycle distribution profile strikingly resembles the distribution of cells treated with puromycin. This result supports the hypothesis that the synthesis of a subset of proteins important for the S-phase progression of CHO-K1 cells might be dependent upon hypusinated eIF-**5A** . Thus the antiproliferative effect of GC7 appears to be related to its interference with the progression of cell cycle, which also provides a possible explanation for the lack of selectivity of GC7 between nontransformed and transformed cell types tested in this study.
? ds

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Set     Items   Description
S1        245   EIF5A OR ((EUCARYOTIC OR EUKARYOTIC) AND INITIATION AND FA-
                CTOR AND 5A)
S2         16   S1 AND APOPTOSIS?
S3         10   S1 AND DHS
S4         84   S1 AND DEOXYHYPUSINE AND SYNTHASE?
S5          1   S4 AND APOPTOSIS?
S6         10   RD S2 (unique items)
S7          5   RD S3 (unique items)
? s s1 and inhibit?
             245  S1
         2009613  INHIBIT?
      S8      89  S1 AND INHIBIT?
? s s8 and antisense?
              89  S8
           34395  ANTISENSE?
      S9       3  S8 AND ANTISENSE?
? rd
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...completed examining records
S10 2 RD (unique items)
? t s10/3,ab/all

10/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13238834 21964436 PMID: 11967308
Complementation of vaccinia virus lacking the double-stranded RNA-binding protein gene E3L by human cytomegalovirus.
Child Stephanie J; Jarrahian Sohail; Harper Victoria M; Geballe Adam P
Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109-1024, USA.
Journal of virology (United States) May 2002, 76 (10) p4912-8, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: AI-26672; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The cellular response to viral infection often includes activation of pathways that shut off protein synthesis and thereby **inhibit** viral replication. In order to enable efficient replication, many viruses carry genes such as the E3L gene of vaccinia virus that counteract these host antiviral pathways. Vaccinia virus from which the E3L gene has been deleted (VVDeltaE3L) is highly sensitive to interferon and exhibits a restricted host range, replicating very inefficiently in many cell types, including human fibroblast and U373MG cells. To determine whether human cytomegalovirus (CMV) has a mechanism for preventing translational shutoff, we evaluated the ability of CMV to complement the deficiencies in replication and protein synthesis associated with VVDeltaE3L. CMV, but not UV-inactivated CMV, rescued VVDeltaE3L late gene expression and replication. Thus, complementation of the VVDeltaE3L defect appears to depend on de novo CMV gene expression and is not likely a result of CMV binding to the cell receptor or of a virion structural protein. CMV rescued VVDeltaE3L late gene expression even in the presence of ganciclovir, indicating that CMV late gene expression is not required for complementation of VVDeltaE3L. The striking decrease in overall translation after infection with VVDeltaE3L was prevented by prior infection with CMV. Finally, CMV blocked both the induction of **eukaryotic initiation factor** 2alpha (eIF2alpha) phosphorylation and activation of RNase L by VVDeltaE3L. These results suggest that CMV has one or more immediate-early or early genes that ensure maintenance of a high protein synthetic capacity during infection by preventing activation of the PKR/eIF2alpha phosphorylation and 2-**5A** oligoadenylate synthetase/RNase L pathways.

10/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07939243 94075396 PMID: 8253832
**Eukaryotic initiation factor 5A** is a cellular target of the human immunodeficiency virus type 1 Rev activation domain mediating trans-activation.
Ruhl M; Himmelspach M; Bahr G M; Hammerschmid F; Jaksche H; Wolff B; Aschauer H; Farrington G K; Probst H; Bevec D; et al
SANDOZ Research Institute, Vienna, Austria.
Journal of cell biology (UNITED STATES) Dec 1993, 123 (6 Pt 1) p1309-20, ISSN 0021-9525 Journal Code: 0375356
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Expression of human immunodeficiency virus type 1 (HIV-1) structural proteins requires the presence of the viral trans-activator protein Rev. Rev is localized in the nucleus and binds specifically to the Rev response element (RRE) sequence in viral RNA. Furthermore, the interaction of the Rev activation domain with a cellular cofactor is essential for Rev function in vivo. Using cross-linking experiments and Biospecific Interaction Analysis (BIA) we identify **eukaryotic initiation factor 5A** (eIF-**5A**) as a cellular **factor** binding specifically to the HIV-1 Rev activation domain. Indirect immunofluorescence studies demonstrate that a significant fraction of eIF-**5A** localizes to the nucleus. We also provide evidence that Rev transactivation is functionally mediated by eIF-**5A** in Xenopus oocytes. Furthermore, we are able to block Rev function in mammalian cells by **antisense inhibition** of eIF-**5A** gene expression. Thus, regulation of HIV-1 gene expression by Rev involves the targeting of RRE-containing RNA to components of the cellular translation **initiation** complex.

s dhs or (deoxyhypusine and synthase) or eif5a or ((eukaryotic or eucaryotic) and initiation and factor and 5a)

| | | |
|---|---|---|
| | 809 | DHS |
| | 189 | DEOXYHYPUSINE |
| | 132457 | SYNTHASE |
| | 39 | EIF5A |
| | 48696 | EUKARYOTIC |
| | 3639 | EUCARYOTIC |
| | 154311 | INITIATION |
| | 1185190 | FACTOR |
| | 10337 | 5A |
| S1 | 1088 | DHS OR (DEOXYHYPUSINE AND SYNTHASE) OR EIF5A OR ((EUKARYOTIC OR EUCARYOTIC) AND INITIATION AND FACTOR AND 5A) |

? s s1 and (antisens? or ribozym?)

| | | |
|---|---|---|
| | 1088 | S1 |
| | 34425 | ANTISENS? |
| | 5971 | RIBOZYM? |
| S2 | 7 | S1 AND (ANTISENS? OR RIBOZYM?) |

? rd
...completed examining records

| | | |
|---|---|---|
| S3 | 4 | RD (unique items) |

? t s3/3,ab/all

3/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13238834 21964436 PMID: 11967308

Complementation of vaccinia virus lacking the double-stranded RNA-binding protein gene E3L by human cytomegalovirus.

Child Stephanie J; Jarrahian Sohail; Harper Victoria M; Geballe Adam P
Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109-1024, USA.

Journal of virology (United States) May 2002, 76 (10) p4912-8, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: AI-26672; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The cellular response to viral infection often includes activation of pathways that shut off protein synthesis and thereby inhibit viral replication. In order to enable efficient replication, many viruses carry genes such as the E3L gene of vaccinia virus that counteract these host antiviral pathways. Vaccinia virus from which the E3L gene has been deleted (VVDeltaE3L) is highly sensitive to interferon and exhibits a restricted host range, replicating very inefficiently in many cell types, including human fibroblast and U373MG cells. To determine whether human cytomegalovirus (CMV) has a mechanism for preventing translational shutoff, we evaluated the ability of CMV to complement the deficiencies in replication and protein synthesis associated with VVDeltaE3L. CMV, but not UV-inactivated CMV, rescued VVDeltaE3L late gene expression and replication. Thus, complementation of the VVDeltaE3L defect appears to depend on de novo CMV gene expression and is not likely a result of CMV binding to the cell receptor or of a virion structural protein. CMV rescued VVDeltaE3L late gene expression even in the presence of ganciclovir, indicating that CMV late gene expression is not required for complementation of VVDeltaE3L. The striking decrease in overall translation after infection with VVDeltaE3L was prevented by prior infection with CMV. Finally, CMV blocked both the induction of **eukaryotic initiation factor** 2alpha (eIF2alpha) phosphorylation and activation of RNase L by VVDeltaE3L. These results suggest that CMV has one or more immediate-early or early genes that ensure maintenance of a high protein synthetic capacity during infection by preventing activation of the

PKR/eIF2alpha phosphorylation and 2-**5A** oligoadenylate synthetase/RNase L pathways.

3/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08669390 96007852 PMID: 7578432
Effect of poly(ADP-ribose) synthetase on the expression of major histocompatibility complex (MHC) class II genes.
Taniguchi T; Ota K; Qu Z; Morisawa K
Medical Research Laboratory, Kochi Medical School, Japan.
Biochimie (FRANCE) 1995, 77 (6) p472-9, ISSN 0300-9084
Journal Code: 1264604
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have studied the effect of poly(ADP-ribose) synthetase on the interferon-gamma (IFN-gamma)-inducible expression of major histocompatibility complex (MHC) class II molecules. We constructed an expression plasmid capable of expressing either a sense RNA (MT-ARS) or an **antisense** RNA (pAS-FL or pAS-5') for poly(ADP-ribose) synthetase. We transfected the plasmid into mouse or human macrophage tumor cells and examined the effect on the expression of MHC class II molecules. The IFN-gamma-inducible expression of MHC class II gene was considerably reduced in transformant clones (A-2, B-2), in which the synthetase was highly expressed, whereas the depletion of the synthetase due to the expression of **antisense** RNA for the synthetase amplified the expression of MHC class II molecules. The results indicate that the level of the synthetase critically regulates the IFN-gamma-inducible MHC class II molecules. Next, we analyzed DNase I hypersensitive sites (**DHS**) of mouse MHC class II, I-A beta gene and found two sites, one in the promoter region and the other one in the first intron. The **DHS** in first intron was less sensitive towards DNase I attack in transformant clones (A-2, B-2) in which the synthetase was synthesized in a large quantity. Thus we constructed two beta-galactosidase reporter genes, one (A beta 2.0kb-lac z) containing the promoter region to a part of the second exon of the class II gene, and the other (A beta pro-lac z) containing the promoter region of the class II gene alone. The expression of the reporter gene was analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) and found that the expression of A beta 2.0kb-lac z was suppressed in the transformant clones (A-2, B-2) relevant to control cells but the expression of A beta pro-lac z was the same level among those cells.(ABSTRACT TRUNCATED AT 250 WORDS)

3/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08416919 95186570 PMID: 7880901
Expression of initiation factor genes in mammalian cells.
Hershey J W
Department of Biological Chemistry, School of Medicine, University of California, Davis 95616.
Biochimie (FRANCE) 1994, 76 (9) p847-52, ISSN 0300-9084
Journal Code: 1264604
Contract/Grant No.: GM22135; GM; NIGMS
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
This review focuses on how cells establish the levels of initiation factors, within the broader context of determining levels of the

translational machinery. Most initiation factor polypeptides are moderately abundant proteins with concentrations approaching those of ribosomes. eIF4A and **eIF5A** are more abundant than ribosomes, whereas eIF4F alpha and eIF2B are considerably less abundant than the other factors. The cloning of cDNAs generates hybridization probes for monitoring the levels and activities of factor mRNAs, and the cloning of their genes is just beginning to provide insight into promoter structures and regulation. Initiation factor gene expression appears to be coordinately regulated in many cases, and preferential synthesis is seen in mitogen-activated T-cells. The gene for eIF2 alpha has been best characterized, and mechanisms that provide for the coordinated synthesis of eIF2 subunits are emerging. Recombinant DNA methods also allow investigators to manipulate the levels of expression of specific factor genes by overexpression or **antisense** repression. Such approaches provide a means to investigate in vivo the mechanisms of action of the initiation factors and their roles in regulating translation rates.

3/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07939243 94075396 PMID: 8253832
**Eukaryotic initiation factor 5A** is a cellular target of the human immunodeficiency virus type 1 Rev activation domain mediating trans-activation.
Ruhl M; Himmelspach M; Bahr G M; Hammerschmid F; Jaksche H; Wolff B; Aschauer H; Farrington G K; Probst H; Bevec D; et al
SANDOZ Research Institute, Vienna, Austria.
Journal of cell biology (UNITED STATES) Dec 1993, 123 (6 Pt 1) p1309-20, ISSN 0021-9525 Journal Code: 0375356
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Expression of human immunodeficiency virus type 1 (HIV-1) structural proteins requires the presence of the viral trans-activator protein Rev. Rev is localized in the nucleus and binds specifically to the Rev response element (RRE) sequence in viral RNA. Furthermore, the interaction of the Rev activation domain with a cellular cofactor is essential for Rev function in vivo. Using cross-linking experiments and Biospecific Interaction Analysis (BIA) we identify **eukaryotic initiation factor 5A** (eIF-**5A**) as a cellular **factor** binding specifically to the HIV-1 Rev activation domain. Indirect immunofluorescence studies demonstrate that a significant fraction of eIF-**5A** localizes to the nucleus. We also provide evidence that Rev transactivation is functionally mediated by eIF-**5A** in Xenopus oocytes. Furthermore, we are able to block Rev function in mammalian cells by **antisense** inhibition of eIF-**5A** gene expression. Thus, regulation of HIV-1 gene expression by Rev involves the targeting of RRE-containing RNA to components of the cellular translation **initiation** complex.
? ds

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Set     Items   Description
S1       1088   DHS OR (DEOXYHYPUSINE AND SYNTHASE) OR EIF5A OR ((EUKARYOT-
            IC OR EUCARYOTIC) AND INITIATION AND FACTOR AND 5A)
S2          7   S1 AND (ANTISENS? OR RIBOZYM?)
S3          4   RD (unique items)
? s s1 and apoptosis?
            1088  S1
          126397  APOPTOSIS?
     S4      17  S1 AND APOPTOSIS?
? rd
...completed examining records
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S5 11 RD (unique items)
? t s5/3,ab/all

5/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12760145 21597923 PMID: 11762979
"Tissue" transglutaminase expression in HIV-infected cells: an enzyme with an antiviral effect?
Amendola A; Rodolfo C; Di Caro A; Ciccosanti F; Falasca L; Piacentini M
Laboratory of Virology, Lazzaro Spallanzani-IRCCS, Rome, Italy.
Annals of the New York Academy of Sciences (United States) Nov 2001, 946 p108-20, ISSN 0077-8923 Journal Code: 7506858
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The cytopathic effect of HIV has been shown to be associated with the induction of **apoptosis** and the inhibition of proliferation of T cells. However, the cellular and molecular mechanisms at the basis of the dramatic immune cell loss caused by HIV in patients suffering from acquired immunodeficient syndrome (AIDS), are not yet fully established. We demonstrated that "tissue" transglutaminase (tTG) gene expression is induced in the immune system of seropositive individuals (peripheral blood mononuclear cells and lymph nodes). tTG is a multifunctional protein involved in a variety of fundamentally important cellular functions, in addition to cell death by **apoptosis**. The presence of high tTG levels in immune-competent cells of HIV+ persons might exert an important role in HIV-infection by influencing viral production. We propose that, in addition to its multiple functions, tTG might interfere with HIV replication by altering the viral mRNA trafficking between the nucleus and the cytoplasm. This effect might be due to its specific interaction with **eIF5A**, a cellular partner of HIV Rev protein, which is essential for HIV replication in immune-competent cells. Given the presence of high tTG levels in HIV+ individuals, it would be of interest to pursue the potential role of this multifunctional protein in the development of strategies aimed at the pharmacologic regulation of HIV production.

5/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11197967 21229963 PMID: 11332455
The role of **eukaryotic initiation factor 5A** in the control of cell proliferation and **apoptosis**.
Caraglia M; Marra M; Giuberti G; D'Alessandro A M; Budillon A; del Prete S; Lentini A; Beninati S; Abbruzzese A
Dipartimento di Biochimica e Biofisica, Seconda Universita di Napoli, Italy.
Amino acids (Austria) 2001, 20 (2) p91-104, ISSN 0939-4451 Journal Code: 9200312
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
In the past years, the attention of scientists has mainly focused on the study of the genetic information and alterations that regulate **eukaryotic** cell proliferation and that lead to neoplastic transformation. An increasing series of data are emerging about the involvement of the **initiation** phase of translational processes in the control of cell proliferation. In this paper we review the novel insights on the biochemical and molecular events leading to the **initiation** and its involvement in cell proliferation and tumourigenesis. We describe the structure, regulation and proposed functions of the **eukaryotic**

**initiation factor 5A** (eIF-**5A** ) focusing the attention on its involvement in the regulation of **apoptosis** and cell proliferation. Moreover, we describe the modulation of its activity (through the reduction of hypusine synthesis) in **apoptosis** induced either by tissue transglutaminase or interferon a. Finally, we propose eIF-**5A** as an additional target of anti-cancer strategies.

5/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10785648 20325068 PMID: 10866791
Modulation of molecular mechanisms involved in protein synthesis machinery as a new tool for the control of cell proliferation.
Caraglia M; Budillon A; Vitale G; Lupoli G; Tagliaferri P; Abbruzzese A
Dipartimento di Biochimica e Biofisica, Seconda Universita di Napoli, Italy.
European journal of biochemistry / FEBS (GERMANY) Jul 2000, 267 (13) p3919-36, ISSN 0014-2956 Journal Code: 0107600
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
In the past years, the attention of scientists has focused mainly on the study of the genetic information and alterations that regulate **eukaryotic** cell proliferation and that lead to neoplastic transformation. All therapeutic strategies against cancer are, to date, directed at DNA either with cytotoxic drugs or gene therapy. Little or no interest has been aroused by protein synthesis mechanisms. However, an increasing body of data is emerging about the involvement of translational processes and factors in control of cell proliferation, indicating that protein synthesis can be an additional target for anticancer strategies. In this paper we review the novel insights on the biochemical and molecular events leading to protein biosynthesis and we describe their involvement in cell proliferation and tumorigenesis. A possible mechanistic explanation is given by the interactions that occur between protein synthesis machinery and the proliferative signal transduction pathways and that are therefore suitable targets for indirect modulation of protein synthesis. We briefly describe the molecular tools used to block protein synthesis and the attempts made at increasing their efficacy. Finally, we propose a new multimodal strategy against cancer based on the simultaneous intervention on protein synthesis and signal transduction.

5/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10664048 20200865 PMID: 10736626
Post-translational modifications of **eukaryotic initiation factor-5A** (eIF-**5A** ) as a new target for anti-cancer therapy.
Caraglia M; Tagliaferri P; Budillon A; Abbruzzese A
Department of Biochemistry and Biophysics F. Cedrangolo, Second University of Naples, Italy.
Advances in experimental medicine and biology (UNITED STATES) 1999, 472 p187-98, ISSN 0065-2598 Journal Code: 0121103
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
**Eukaryotic** translation **initiation factor 5A** (eIF-**5A** ) is the only cell protein that contains the unusual basic amino acid hypusine [N epsilon-(4-amino-2-hydroxybutyl)lysine]. Hypusine is formed by the transfer of the butylamine portion from spermidine to the

epsilon-amino group of a specific lysine residue of eIF-**5A** precursor and the subsequent hydroxylation at carbon 2 of the incoming 4-aminobutyl moiety. Agents that reduce cell hypusine levels inhibit the growth of mammalian cells. These observations suggest that hypusine is crucial for proliferation and transformation of **eukaryotic** cells. Here we have studied whether the inhibition of hypusine synthesis can potentiate the anti-cancer activity of the anti-tumour agents interferon-alpha (IFN alpha) and cytosine arabinoside (ara-C). We have found that IFN alpha increased epidermal growth **factor** receptor (EGF-R) expression, but reduced S phase and proliferative marker expression in human epidermoid KB cells and that this effect was antagonised by epidermal growth **factor** (EGF). Growth inhibition induced by IFN alpha was paralleled by decreased hypusine synthesis and, when EGF counteracted anti-proliferative effects, a reconstitution of hypusine levels was recorded. We also studied the effects of IFN alpha on the cytotoxicity of the recombinant toxin TP40 which inhibits elongation **factor** 2, another step of protein synthesis, through EGF-R binding and internalisation; IFN alpha induced an about 27-fold increase of TP40 cytotoxicity in KB cells. Ara-C, another antineoplastic agent commonly used in haematologic malignancies, induced both **apoptosis** and iron depletion in human acute myeloid leukaemic cells. The combination of ara-C and of the iron chelator desferioxamine, a strong inhibitor of hypusine synthesis, had a synergistic activity on **apoptosis** in these cells. The data strongly suggest that the post-translational modifications of eIF-**5A** could be a suitable target for the potentiation of the activity of anti-cancer agents.

5/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10663528 20202622 PMID: 10737792

Expression analysis with oligonucleotide microarrays reveals that MYC regulates genes involved in growth, cell cycle, signaling, and adhesion.

Coller H A; Grandori C; Tamayo P; Colbert T; Lander E S; Eisenman R N; Golub T R

Center for Genome Research, Whitehead Institute for Biomedical Research, Cambridge, MA 02139, USA. hcoller@fhcrc.org

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Mar 28 2000, 97 (7) p3260-5, ISSN 0027-8424
Journal Code: 7505876

Contract/Grant No.: CA20525; CA; NCI; CA75125; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

MYC affects normal and neoplastic cell proliferation by altering gene expression, but the precise pathways remain unclear. We used oligonucleotide microarray analysis of 6,416 genes and expressed sequence tags to determine changes in gene expression caused by activation of c-MYC in primary human fibroblasts. In these experiments, 27 genes were consistently induced, and 9 genes were repressed. The identity of the genes revealed that MYC may affect many aspects of cell physiology altered in transformed cells: cell growth, cell cycle, adhesion, and cytoskeletal organization. Identified targets possibly linked to MYC's effects on cell growth include the nucleolar proteins nucleolin and fibrillarin, as well as the **eukaryotic initiation factor 5A**. Among the cell cycle genes identified as targets, the G1 cyclin D2 and the cyclin-dependent kinase binding protein CksHs2 were induced whereas the cyclin-dependent kinase inhibitor p21(Cip1) was repressed. A role for MYC in regulating cell adhesion and structure is suggested by repression of genes encoding the extracellular matrix proteins fibronectin and collagen, and the cytoskeletal protein tropomyosin. A possible mechanism for MYC-mediated **apoptosis** was revealed by identification of the tumor necrosis **factor** receptor associated protein TRAP1 as a MYC target.

Finally, two immunophilins, peptidyl-prolyl cis-trans isomerase F and FKBP52, the latter of which plays a role in cell division in Arabidopsis, were up-regulated by MYC. We also explored pattern-matching methods as an alternative approach for identifying MYC target genes. The genes that displayed an expression profile most similar to endogenous Myc in microarray-based expression profiling of myeloid differentiation models were highly enriched for MYC target genes.

5/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09944365 98378101 PMID: 9714299
Antiretroviral effects of deoxyhypusyl hydroxylase inhibitors: a hypusine-dependent host cell mechanism for replication of human immunodeficiency virus type 1 (HIV-1).
Andrus L; Szabo P; Grady R W; Hanauske A R; Huima-Byron T; Slowinska B; Zagulska S; Hanauske-Abel H M
The New York Blood Center, NY, USA.
Biochemical pharmacology (ENGLAND) Jun 1 1998, 55 (11) p1807-18, ISSN 0006-2952 Journal Code: 0101032
Contract/Grant No.: AI34773; AI; NIAID; PO1AG00541; AG; NIA; R37AG08707; AG; NIA; +
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The HIV-1 protein Rev, critical for translation of incompletely spliced retroviral mRNAs encoding capsid elements, requires a host cell protein termed "**eukaryotic initiation factor 5A**" (eIF-**5A** ). This is the only protein containing hypusine, a lysine-derived hydroxylated residue that determines its proposed bioactivity, the translation of a subset of cellular mRNAs controlling G1-to-S transit of the cell cycle. We postulated that inhibiting the hypusine-forming deoxyhypusyl hydroxylase (DOHH) should, by depleting **eukaryotic initiation factor 5A** , compromise Rev function and thus reduce HIV-1 multiplication. We now report that the alpha-hydroxypyridones, specifically mimosine, a natural product, and deferiprone, an experimental drug, inhibited deoxyhypusyl hydroxylase in T-lymphocytic and promonocytic cell lines and, in a concentration-dependent manner, suppressed replication of HIV-1. However, the alpha-hydroxypyridones did not affect the formation of unspliced or multiply spliced HIV-1 transcripts. Rather, these agents caused Rev-dependent incompletely spliced HIV-1 mRNA such as gag, but not cellular "housekeeping" mRNAs, to disappear from polysomes. Consequently, alpha-hydroxypyridone-mediated depletion of eIF-**5A** decreased biosynthesis of structural HIV-1 protein encoded by gag, measured as p24, whereas the induced formation of cellular protein like tumor necrosis **factor** alpha remained unaffected. By interfering with the translation of incompletely spliced retroviral mRNAs, these compounds restrict HIV-1 to the early, nongenerative phase of its reproductive cycle. In the inducibly HIV-1 expressing T-cell line ACH-2, the deoxyhypusyl hydroxylase inhibitors triggered extensive **apoptosis** , particularly of cells that actively produce HIV-1. Selective suppression of retroviral protein biosynthesis and preferential **apoptosis** of retrovirally infected cells by alpha-hydroxypyridones point to a novel mode of antiretroviral action.

5/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09689842 98113151 PMID: 9442029
Identification of the **eukaryotic initiation factor 5A** as a retinoic acid-stimulated cellular binding partner for tissue transglutaminase II.

Singh U S; Li Q; Cerione R
Department of Pharmacology, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853, USA.
Journal of biological chemistry (UNITED STATES) Jan 23 1998, 273 (4) p1946-50, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: EY06429; EY; NEI; GM40654; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
GTP-binding protein/transglutaminases (tissue transglutaminases or TGases) have been implicated in a variety of cellular processes including retinoic acid (RA)-induced **apoptosis**. Recently, we have shown that RA activates TGases as reflected by stimulated GTP binding, increased membrane association, and stimulated phosphoinositide lipid turnover. This prompted us to search for cellular proteins that bind TGases in a RA-stimulated manner. In this report, we show that the **eukaryotic initiation factor** (eIF-**5A** ), a protein that is essential for cell viability, perhaps through effects on protein synthesis and/or RNA export, associates with the TGase in vivo. The interaction between eIF-**5A** and TGase is specific for the GDP-bound form of the TGase and is not detected when the TGase is pre-loaded with GTP gamma S. The TGase-eIF-**5A** interaction also is promoted by Ca2+, Mg2+, and RA treatment of HeLa cells. In the presence of retinoic acid, millimolar levels of Ca2+ are no longer required for the TGase-eIF-**5A** interaction. Nocodazole treatment, which blocks the cell cycle at mitosis (M phase), strongly inhibits the interaction between eIF-**5A** and cytosolic TGase. The interaction between TGase and eIF-**5A** and its sensitivity to the nucleotide-occupied state of the TGase provides a potentially interesting connection between RA signaling and protein synthesis and/or RNA trafficking activities.

5/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09637936 98060742 PMID: 9396730
Excess putrescine accumulation inhibits the formation of modified **eukaryotic initiation factor 5A** (eIF-**5A**) and induces **apoptosis**.
Tome M E; Fiser S M; Payne C M; Gerner E W
Department of Radiation Oncology, Arizona Health Sciences Center, University of Arizona, Tucson, AZ 85724, USA.
Biochemical journal (ENGLAND) Dec 15 1997, 328 ( Pt 3) p847-54, ISSN 0264-6021 Journal Code: 2984726R
Contract/Grant No.: CA-23074; CA; NCI; CA-30052; CA; NCI; CA-72008; CA; NCI; +
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
DH23A cells, an alpha-difluoromethylornithine-resistant variant of the parental hepatoma tissue culture cells, express high levels of stable ornithine decarboxylase. Aberrantly high expression of ornithine decarboxylase results in a large accumulation of endogenous putrescine and increased **apoptosis** in DH23A cells when alpha-difluoromethylornithine is removed from the culture. Treatment of DH23A cells with exogenous putrescine in the presence of alpha-difluoromethylornithine mimics the effect of drug removal, suggesting that putrescine is a causative agent or trigger of **apoptosis**. Accumulation of excess intracellular putrescine inhibits the formation of hypusine in vivo, a reaction that proceeds by the transfer of the butylamine moiety of spermidine to a lysine residue in **eukaryotic initiation factor 5A** (eIF-**5A**).
Treatment of DH23A cells with diaminoheptane, a competitive inhibitor of

the post-translational modification of eIF-**5A** , causes both the suppression of eIF-**5A** modification in vivo and induction of **apoptosis** . These data support the hypothesis that rapid degradation of ornithine decarboxylase is a protective mechanism to avoid cell toxicity from putrescine accumulation. Further, these data suggest that suppression of modified eIF-**5A** formation is one mechanism by which cells may be induced to undergo **apoptosis**.

5/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09523055 97430999 PMID: 9285098
Cellular **eukaryotic initiation factor 5A** content as a mediator of polyamine effects on growth and **apoptosis**.
Tome M E; Gerner E W
Department of Biochemistry, University of Arizona, Tucson 85724, USA.
Biological signals (SWITZERLAND) May-Jun 1997, 6 (3) p150-6, ISSN 1016-0922 Journal Code: 9210083
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The polyamines are essential for **eukaryotic** cell growth. One of the most critical effects of polyamines on cell growth is the availability of spermidine for the post-translational modification of eIF-**5A**. Because hypusine-containing eIF-**5A** is necessary for cell proliferation, depletion of cellular polyamines suppresses growth by depleting cellular modified eIF-**5A** content. Excess putrescine accumulations in DH23A/b cells induces **apoptosis** and suppresses the formation of hypusine-containing eIF-**5A** . Treatment of DH23A/b cells with diaminoheptane also suppresses modified eIF-**5A** formation and induces **apoptosis**. These data suggest that suppression of modified eIF-**5A** formation may play a role in putrescine-induced **apoptosis** as well.

5/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08959502 96312847 PMID: 8701983
Ductular reaction after submassive necrosis in humans. Special emphasis on analysis of ductular hepatocytes.
Demetris A J; Seaberg E C; Wennerberg A; Ionellie J; Michalopoulos G
Pittsburgh Transplant Institute, Department of Pathology, University of Pittsburgh Medical Center, Pennsylvania, USA.
American journal of pathology (UNITED STATES) Aug 1996, 149 (2) p439-48, ISSN 0002-9440 Journal Code: 0370502
Contract/Grant No.: 1 RO1 DK49615-01A1; DK; NIDDK; RO1 2R37CA30241-15; CA ; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The ductular reaction to acute submassive necrosis was studied in human livers removed at the time of orthotopic liver transplantation. Single, double, and triple immunohistochemical labeling in combination with morphometry was used to analyze the phenotype and proliferative and apoptotic rates of various epithelial cell compartments. These were divided on the basis of immunohistochemistry and morphology into three subtypes: 1) CK19+/AE1+ mature bile duct epithelium, 2) HEP-PAR+ mature hepatocytes (HEPs), and 3) CK19+/AE1+ ductular hepatocyte (DH) cells lying at the interface between the portal tract connective tissue and the hepatic lobules. Cycling cells were defined as those showing Ki-67+ (MIB-1) nuclear

labeling. Apoptotic cells were identified with in situ labeling using the terminal deoxynucleotidyl transferase-mediated dUTP-digoxigenin nick end labeling assay. Special emphasis was placed on **DHs** that appeared at the interface between the portal tracts and hepatic lobules. During the recovery phase from submassive hepatic necrosis, subtraction of the rate of cell death from the proliferative index shows that all of the epithelial compartments experience a net increase in the number of cells. The highest proliferation rate occurs in the **DHs**, which is significantly ($P < 0.0001$) higher than the proliferation rate seen in either the HEP or bile duct epithelium compartments. Immunohistochemical analysis of the highly proliferative DH compartment shows it to be a heterogeneous population with unique phenotypic features. Like epithelial cells in the ductal plate of fetal liver and cholangiocarcinomas, **DHs** are positioned on a laminin-rich matrix and focally express vimentin and Lewis(x) and show up-regulation of bcl-2 and type IV collagenase. However, unlike ductal plate cells, **DHs** are CD34 and alpha-fetoprotein negative. Although a subpopulation of **DHs** share phenotypic features with mature bile duct epithelium (AE1/cytokeratin 19 and type IV collagenase positive) or HEP (HEP-PAR, albumin, and alpha-1-antitrypsin positive), they are also clearly separate from both populations; **DHs** are negative or only weakly stain for glutathione-S-transferase-pi and are type IV collagenase positive. Moreover, occasional **DHs** also co-expressed HEP-PAR or alpha-1-antitrypsin and AE1, indicative of both hepatocyte and ductular differentiation. These findings suggest that **DHs** seen in human livers after submassive necrosis may represent a transient amplifying population arising from a progenitor population located in or near the canals of Herring. In addition, injured hepatocytes can express cytokeratin 19 and AE1, which normally are biliary intermediate filaments.

5/3,AB/11 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)


13526822 BIOSIS NO.: 200200155643
Effects of N1-guanyl-1,7-diaminoheptane, an inhibitor of **deoxyhypusine synthase**, on endothelial cell growth, differentiation and **apoptosis**.
AUTHOR: Joe Young Ae(a); Lee Yoon; Kim Hyun-Kyung; Kim You Young; Park Myung Hee
AUTHOR ADDRESS: (a)Cancer Research Institute, The Catholic University of Korea, 505 Banpo-dong, Seocho-ku, Seoul, 137-701**South Korea
JOURNAL: Molecular Biology of the Cell 11 (Supplement):p455a Dec., 2000
MEDIUM: print
CONFERENCE/MEETING: 40th American Society for Cell Biology Annual Meeting San Francisco, CA, USA December 09-13, 2000
ISSN: 1059-1524
RECORD TYPE: Citation
LANGUAGE: English
2000
? ds

| Set | Items | Description |
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| S2 | 7 | S1 AND (ANTISENS? OR RIBOZYM?) |
| S3 | 4 | RD (unique items) |
| S4 | 17 | S1 AND APOPTOSIS? |
| S5 | 11 | RD (unique items) |

? s s1 and spermidin?

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|---|---|---|
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| | 14336 | SPERMIDIN? |
| S6 | 105 | S1 AND SPERMIDIN? |

? rd

...examined 50 records (50)
...examined 50 records (100)
...completed examining records
S7 65 RD (unique items)
? s s7 and apoptosis?
65 S7
126397 APOPTOSIS?
S8 3 S7 AND APOPTOSIS?
? t s8/3/all

8/3/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10664048 20200865 PMID: 10736626
Post-translational modifications of **eukaryotic initiation factor-5A** (eIF-**5A**) as a new target for anti-cancer therapy.
Caraglia M; Tagliaferri P; Budillon A; Abbruzzese A
Department of Biochemistry and Biophysics F. Cedrangolo, Second University of Naples, Italy.
Advances in experimental medicine and biology (UNITED STATES) 1999, 472 p187-98, ISSN 0065-2598 Journal Code: 0121103
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

8/3/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09637936 98060742 PMID: 9396730
Excess putrescine accumulation inhibits the formation of modified **eukaryotic initiation factor 5A** (eIF-**5A**) and induces **apoptosis**.
Tome M E; Fiser S M; Payne C M; Gerner E W
Department of Radiation Oncology, Arizona Health Sciences Center, University of Arizona, Tucson, AZ 85724, USA.
Biochemical journal (ENGLAND) Dec 15 1997, 328 ( Pt 3) p847-54, ISSN 0264-6021 Journal Code: 2984726R
Contract/Grant No.: CA-23074; CA; NCI; CA-30052; CA; NCI; CA-72008; CA; NCI; +
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

8/3/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09523055 97430999 PMID: 9285098
Cellular **eukaryotic initiation factor 5A** content as a mediator of polyamine effects on growth and **apoptosis**.
Tome M E; Gerner E W
Department of Biochemistry, University of Arizona, Tucson 85724, USA.
Biological signals (SWITZERLAND) May-Jun 1997, 6 (3) p150-6, ISSN 1016-0922 Journal Code: 9210083
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
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Set     Items   Description
S1       1088   DHS OR (DEOXYHYPUSINE AND SYNTHASE) OR EIF5A OR ((EUKARYOT-
            IC OR EUCARYOTIC) AND INITIATION AND FACTOR AND 5A)
S2          7   S1 AND (ANTISENS? OR RIBOZYM?)
S3          4   RD (unique items)
S4         17   S1 AND APOPTOSIS?
S5         11   RD (unique items)
S6        105   S1 AND SPERMIDIN?
S7         65   RD (unique items)
S8          3   S7 AND APOPTOSIS?
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Processing
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                1  DIAMO
             7556  PROPANE
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             1088  S1
             5375  DIAMINO
             7556  PROPANE
     S11        0  S1 AND DIAMINO AND PROPANE
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             5375  DIAMINO
             2219  BUTANE
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            12909  PUTRESCINE
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     S15       19  S14 AND PY<2002
? t s15/3,ab/all

 15/3,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10993903   20558097   PMID: 11104695
   Human deoxyhypusine synthase : interrelationship between
binding of NAD and substrates.
  Lee C H; Park M H
  Building 30, Room 211, Oral and Pharyngeal Cancer Branch, National
Institute of Dental and Craniofacial Research, National Institutes of
Health, Bethesda, MD 20892-4340, USA.
  Biochemical journal (ENGLAND)   Dec 15 2000, 352 Pt 3  p851-7,
ISSN 0264-6021   Journal Code: 2984726R
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  Deoxyhypusine synthase catalyses the NAD-dependent transfer
of the butylamine moiety from the polyamine, spermidine, to a specific
lysine residue of a single cellular protein, eukaryotic translation-
initiation factor 5A (eIF5A) precursor. The native
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enzyme exists as a tetramer of four identical subunits and contains four binding sites for NAD. The binding of spermidine and NAD was studied by a filtration assay. [(3)H]Spermidine binding to the enzyme was not detectable alone or in the presence of the **eIF5A** precursor, but was detected only in the presence of NAD or NADH, suggesting that a NAD/NADH-induced conformational change is required for the binding of spermidine. A strong NAD-dependent binding was also observed with a spermidine analogue, N(1)-guanyl-1, 7-diamino[(3)H]heptane (GC(7)), but not with [(14)C] **putrescine** or [(14)C]spermine. Although [(3)H]NAD binding to the enzyme occurred in the absence of spermidine, its affinity for the enzyme was markedly enhanced by spermidine, GC(7) and also by the **eIF5A** precursor. The maximum binding for NAD and spermidine was estimated to be approximately 4 molecules each/enzyme tetramer. The dependence of spermidine binding on NAD and the modulation of binding of NAD by spermidine and the **eIF5A** precursor suggest intricate relationships between the binding of cofactor and the substrates, and provide new insights into the reaction mechanism.

15/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10614452 20125598 PMID: 10657256
*Changes in gene expression in response to polyamine depletion indicates selective stabilization of mRNAs.*
*Veress I; Haghighi S; Pulkka A; Pajunen A*
Department of Biochemistry, University of Oulu, P.O. Box 400, FIN-90571 Oulu, Finland.
Biochemical journal (ENGLAND) Feb 15 **2000**, 346 Pt 1 p185-91, ISSN 0264-6021 Journal Code: 2984726R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We used differential display analysis to identify mRNAs responsive to changes in polyamine synthesis. As an overproducing model we used the kidneys of transgenic hybrid mice overexpressing ornithine decarboxylase and S-adenosylmethionine decarboxylase, two key enzymes in polyamine biosynthesis. To identify mRNAs that respond to polyamine starvation, we treated Rat-2 cells with alpha-difluoromethylornithine, a specific inhibitor of polyamine biosynthesis. We isolated 41 partial cDNA clones, representing 37 differentially expressed mRNAs. Of these, 15 have similarity with known genes, five appear to be similar to reported expressed sequence tags and seventeen clones were novel sequences. Of the 35 mRNAs expressed differentially after alpha-difluoromethylornithine treatment, 26 were up-regulated. The expression of only three mRNAs was altered in the transgenic animals and all three were down-regulated. Determination of mRNA half-life of three of the mRNAs up-regulated in response to polyamine depletion revealed that the accumulation results from stabilization of the messages. Because most of the transcripts identified from Rat-2 cells suffering polyamine starvation were accumulated, we conclude that polyamine depletion, while blocking cell growth, is stabilizing mRNAs. This may be due to the lack of spermidine for post-translational modification of the **eukaryotic initiation factor 5A** , which plays a major role in mRNA turnover. The coupling of mRNA stabilization with cell-growth arrest in response to polyamine starvation provides cells with an economical way to resume growth after recovery from polyamine deficiency.

15/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10487785 20011401 PMID: 10542236

**Deoxyhypusine synthase** from tobacco. cDNA isolation, characterization, and bacterial expression of an enzyme with extended substrate specificity.

Ober D; Hartmann T
Institut fur Pharmazeutische Biologie der Technischen Universitat Braunschweig, Mendelssohnstrasse 1, D-38106 Braunschweig, Germany.

Journal of biological chemistry (UNITED STATES) Nov 5 **1999**, 274 (45) p32040-7, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

**Deoxyhypusine synthase** catalyzes the formation of a **deoxyhypusine** residue in the translation **eukaryotic initiation factor 5A** (**eIF5A**) precursor protein by transferring an aminobutyl moiety from spermidine onto a conserved lysine residue within the **eIF5A** polypeptide chain. This reaction commences the activation of the **initiation factor** in fungi and vertebrates. A mechanistically identical reaction is known in the biosynthetic pathway leading to pyrrolizidine alkaloids in plants. **Deoxyhypusine synthase** from tobacco was cloned and expressed in active form in Escherichia coli. It catalyzes the formation of a **deoxyhypusine** residue in the tobacco **eIF5A** substrate as shown by gas chromatography coupled with a mass spectrometer. The enzyme also accepts free **putrescine** as the aminobutyl acceptor, instead of lysine bound in the **eIF5A** polypeptide chain, yielding homospermidine. Conversely, it accepts homospermidine instead of spermidine as the aminobutyl donor, whereby the reactions with **putrescine** and homospermidine proceed at the same rate as those involving the authentic substrates. The conversion of **deoxyhypusine synthase**-catalyzed **eIF5A** deoxyhypusinylation pinpoints a function for spermidine in plant metabolism. Furthermore, and quite unexpectedly, the substrate spectrum of **deoxyhypusine synthase** hints at a biochemical basis behind the sparse and skew occurrence of both homospermidine and its pyrrolizidine derivatives across distantly related plant taxa.

15/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10408253 99407273 PMID: 10476066

Cloning and expression of a cDNA encoding homospermidine **synthase** from Senecio vulgaris (Asteraceae) in Escherichia coli.

Kaiser A
Institute of Pharmaceutical Biology, Braunschweig, Germany. akaiser@parasit.meb.uni-bonn.de

Plant journal : for cell and molecular biology (ENGLAND) Jul **1999**, 19 (2) p195-201, ISSN 0960-7412 Journal Code: 9207397
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The enzyme homospermidine **synthase** catalyzes the NAD+-dependent conversion of 2 mol **putrescine** into homospermidine. Instead of **putrescine**, spermidine can substitute for the first **putrescine** moiety in plants, in which case diaminopropane instead of ammonia is released. The enzyme facilitates the formation of the 'uncommon' polyamine homospermidine which is an important precursor in the biosynthesis of pyrrolizidine alkaloids. The first plant homospermidine **synthase** was purified to apparent chemical homogenity from the root tissue culture Senecio vernalis (Asteraceae) (Bottcher et al. 1994, Can. J. Chem. 72, 80-85; Ober 1997, Dissertation). Four endopeptidase LysC fragments were sequenced from the purified protein. With the aid of degenerate primers against these peptides, a cDNA encoding homospermidine **synthase** was

now cloned and characterized from Senecio vulgaris. The nucleotide sequence of the cloned cDNA revealed an open reading frame of 1155-base pairs containing 385 amino acids with a predicted Mr of 44500. GenBank research revealed that the deduced amino acid sequence shows 59% identity to human **deoxyhypusine synthase**. The homospermidine **synthase** encoding cDNA was subcloned into the expression vector pet15b and overexpressed in E. coli. The recombinant enzyme formed upon expression catalyzed homospermidine synthesis.

15/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09637936 98060742 PMID: 9396730
Excess **putrescine** accumulation inhibits the formation of modified **eukaryotic initiation factor 5A** (eIF-**5A**) and induces apoptosis.
Tome M E; Fiser S M; Payne C M; Gerner E W
Department of Radiation Oncology, Arizona Health Sciences Center, University of Arizona, Tucson, AZ 85724, USA.
Biochemical journal (ENGLAND) Dec 15 **1997**, 328 ( Pt 3) p847-54, ISSN 0264-6021 Journal Code: 2984726R
Contract/Grant No.: CA-23074; CA; NCI; CA-30052; CA; NCI; CA-72008; CA; NCI; +
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
DH23A cells, an alpha-difluoromethylornithine-resistant variant of the parental hepatoma tissue culture cells, express high levels of stable ornithine decarboxylase. Aberrantly high expression of ornithine decarboxylase results in a large accumulation of endogenous **putrescine** and increased apoptosis in DH23A cells when alpha-difluoromethylornithine is removed from the culture. Treatment of DH23A cells with exogenous **putrescine** in the presence of alpha-difluoromethylornithine mimics the effect of drug removal, suggesting that **putrescine** is a causative agent or trigger of apoptosis. Accumulation of excess intracellular **putrescine** inhibits the formation of hypusine in vivo, a reaction that proceeds by the transfer of the butylamine moiety of spermidine to a lysine residue in **eukaryotic initiation factor 5A** (eIF-**5A**). Treatment of DH23A cells with diaminoheptane, a competitive inhibitor of the post-translational modification of eIF-**5A** , causes both the suppression of eIF-**5A** modification in vivo and induction of apoptosis. These data support the hypothesis that rapid degradation of ornithine decarboxylase is a protective mechanism to avoid cell toxicity from **putrescine** accumulation. Further, these data suggest that suppression of modified eIF-**5A** formation is one mechanism by which cells may be induced to undergo apoptosis.

15/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09523055 97430999 PMID: 9285098
Cellular **eukaryotic initiation factor 5A** content as a mediator of polyamine effects on growth and apoptosis.
Tome M E; Gerner E W
Department of Biochemistry, University of Arizona, Tucson 85724, USA.
Biological signals (SWITZERLAND) May-Jun **1997**, 6 (3) p150-6, ISSN 1016-0922 Journal Code: 9210083
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM

Record type: Completed
The polyamines are essential for **eukaryotic** cell growth. One of the most critical effects of polyamines on cell growth is the availability of spermidine for the post-translational modification of eIF-**5A**. Because hypusine-containing eIF-**5A** is necessary for cell proliferation, depletion of cellular polyamines suppresses growth by depleting cellular modified eIF-**5A** content. Excess **putrescine** accumulations in DH23A/b cells induces apoptosis and suppresses the formation of hypusine-containing eIF-**5A** . Treatment of DH23A/b cells with diaminoheptane also suppresses modified eIF-**5A** formation and induces apoptosis. These data suggest that suppression of modified eIF-**5A** formation may play a role in **putrescine**-induced apoptosis as well.

15/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09423703 97306062 PMID: 9163530
Biochemical and immunological characterization of **deoxyhypusine synthase** purified from the yeast Saccharomyces carlsbergensis.
Abid M R; Sasaki K; Titani K; Miyazaki M
Department of Molecular Biology, School of Science, Nagoya University, Chikusa-ku.
Journal of biochemistry (JAPAN) Apr **1997**, 121 (4) p769-78, ISSN 0021-924X Journal Code: 0376600
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
**Deoxyhypusine synthase** catalyzes the NAD+-dependent formation of **deoxyhypusine** in the eIF-5A precursor protein by transferring the 4-aminobutyl moiety of spermidine. This enzyme has recently been shown to be essential for cell viability and growth of yeast [Sasaki, K., Abid, M.R., and Miyazaki, M. (1996) FEBS Lett. 384, 151 154]. We have purified and characterized the enzyme from the yeast Saccharomyces carlsbergensis. The yeast and recombinant enzymes had a specific activity of 1.21 to 1.26 pmol per min per pmol of protein, and recognized both the eIF-5A precursor proteins almost equally as judged from their similar K(m) and V(max) values. Size exclusion chromatography and SDS-PAGE indicated that the active form of the enzyme is a homotetramer consisting of 43-kDa subunits. The enzyme showed a strict specificity for its substrates, NAD+, spermidine and eIF-5A precursor protein. Among all the substrates tested, only NAD+ showed a protective effect against heat inactivation of the enzyme suggesting that NAD+ initiates some conformational change in the enzyme. NADH exhibited a strong non-competitive inhibition (product inhibition). Unexpectedly, FAD, FMN, and riboflavin showed a moderate competitive inhibition. The competitive inhibition by diamines was maximal with compounds resembling spermidine in carbon chain length. 1,3-Diaminopropane inhibited the enzyme strongly in a competitive manner (product inhibition). On the other hand, **putrescine** did not inhibit the enzyme or act as a substrate. A polyclonal antibody raised against the yeast recombinant enzyme specifically inhibited **deoxyhypusine synthase** activity. The cross-reactivity (by Western blotting) of this antibody with the crude extracts varied depending on the source, indicating species specificity.

15/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08957689 96319714 PMID: 8697449
Effects of inhibitors of **deoxyhypusine synthase** on the differentiation of mouse neuroblastoma and erythroleukemia cells.
Chen Z P; Yan Y P; Ding Q J; Knapp S; Potenza J A; Schugar H J; Chen K Y
Department of Chemistry, State University of New Jersey, Piscataway

08855-0939, USA.
Cancer letters (IRELAND) Aug 2 **1996**, 105 (2) p233-9, ISSN 0304-3835 Journal Code: 7600053
Contract/Grant No.: CA49695; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Deoxyhpusine **synthase** catalyzes the conversion of lysine to **deoxyhypusine** residue on the **eukaryotic initiation factor 5A** (eIF-**5A** ) precursor using spermidine as the substrate. Subsequent hydroxylation of the **deoxyhypusine** residue completes hypusine formation on eIF-**5A**. Polyamines (**putrescine**, spermidine, and spermine) have been implicated in tumor growth and differentiation. Because **deoxyhypusine**/hypusine formation is one of the most specific polyamine-dependent biochemical events, we decided to use N1-guanyl-1,7-diaminoheptane (GC7), a potent inhibitor for **deoxyhypusine synthase** , to assess the role of hypusine formation on tumor growth and differentiation. GC7 suppressed the growth of N2a mouse neuroblastoma cells and DS19 murine erythroleukemia cells at micromolar concentrations. However, within a narrow concentration range, GC7 could promote the differentiation of mouse neuroblastoma cells in the presence of suboptimal amount of dibutyryl cAMP. In contrast, GC7 blocked the differentiation of DS19 cells induced with hexamethylene bisacetamide. Polyamine depletion by difluoromethyl ornithine (DFMO) has previously been shown to promote differentiation of neuroblastoma cells but inhibits erythrodifferentiation. Since our studies demonstrated that GC7 mimics the action of DFMO on tumor differentiation, it is likely that the effect of DFMO on tumor differentiation is mediated by hypusine formation and that GC7 represents a more specific inhibitor that can alter the differentiation program in certain tumor cells.

15/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08314523 95071231 PMID: 7980394
The role of hypusine depletion in cytostasis induced by S-adenosyl-L-methionine decarboxylase inhibition: new evidence provided by 1-methylspermidine and 1,12-dimethylspermine.
Byers T L; Lakanen J R; Coward J K; Pegg A E
Department of Cell and Molecular Physiology, M.S. Hershey Medical Center, Hershey, PA 17033.
Biochemical journal (ENGLAND) Oct 15 **1994**, 303 ( Pt 2) p363-8, ISSN 0264-6021 Journal Code: 2984726R
Contract/Grant No.: CA-37606; CA; NCI; GM-26290; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The abilities of the natural polyamines, spermidine and spermine, and of the synthetic analogues, 1-methylspermidine and 1,12-dimethylspermine, to reverse the effects of the S-adenosyl-L-methionine decarboxylase inhibitor 5'-([(Z)-4-aminobut-2-enyl]methylamino)-5'-deoxyadenosine (AbeAdo) on L1210-cell growth were studied. L1210 cells were exposed to AbeAdo for 12 days to induce cytostasis and then exposed to spermidine, spermine, 1-methylspermidine or 1,12-dimethylspermine in the continued presence of AbeAdo. AbeAdo-induced cytostasis was overcome by the natural polyamines, spermidine and spermine. The cytostasis was also reversed by 1-methylspermidine. 1,12-Dimethylspermine had no effect on the AbeAdo-induced cytostasis of chronically treated cells, although it was active in permitting growth of cells treated with the ornithine decarboxylase inhibitor, alpha-difluoromethylornithine. The initial 12-day exposure to AbeAdo elevated intracellular **putrescine** levels, depleted

intracellular spermidine and spermine, and resulted in the accumulation of unmodified **eukaryotic** translation **initiation factor** **5A** (eIF-**5A** ). Exposure of these cells to exogenous spermidine, which is the natural substrate for **deoxyhypusine synthase**, resulted in a decrease in the unmodified eIF-**5A** content. 1-Methylspermidine, which was found to be a substrate of **deoxyhypusine synthase** in vitro, also decreased the levels of unmodified eIF-**5A** in the AbeAdo-treated cells. Although spermine is not a substrate of **deoxyhypusine synthase** , spermine was converted into spermidine in the L1210 cells, and spermine addition to AbeAdo-treated cells resulted in the appearance of both intracellular spermine and spermidine and in the decrease in unmodified eIF-**5A**. Exogenous 1,12-dimethylspermine, which was not metabolized to spermine or to 1-methylspermidine and was not a substrate of **deoxyhypusine** **synthase** in vitro, did not decrease levels of unmodified eIF-**5A** . The finding that AbeAdo-induced cytostasis was only reversed by polyamines and polyamine analogues that result in the formation of hypusine or an analogue in eIF-**5A** is consistent with the hypothesis [Byers, Wiest, Wechter and Pegg (1993) Biochem. J. 290, 115-121] that AbeAdo-induced cytostasis is due to the depletion of the hypusine-containing form of eIF-**5A** , which is secondary to the depletion of spermidine by inhibition of S-adenosyl-L-methionine decarboxylase.

15/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08292540 95050547 PMID: 7961711

Antiproliferative effects of inhibitors of **deoxyhypusine** **synthase** . Inhibition of growth of Chinese hamster ovary cells by guanyl diamines.

Park M H; Wolff E C; Lee Y B; Folk J E

Enzyme Chemistry Section, NIDR, National Institutes of Health, Bethesda, Maryland 20892.

Journal of biological chemistry (UNITED STATES) Nov 11 **1994**, 269 (45) p27827-32, ISSN 0021-9258 Journal Code: 2985121R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Certain guanyl diamines are effective inhibitors of **deoxyhypusine** **synthase** (Jakus, J., Wolff, E. C., Park, M. H., and Folk, J. E. (1993) J. Biol. Chem. 268, 13151-13159), the first enzyme involved in the biosynthesis of the unusual amino acid hypusine (N epsilon-(4-amino-2-hydroxybutyl)lysine). Evidence that hypusine is implicated in cell growth prompted this study of the cellular effects of these inhibitors. In Chinese hamster ovary (CHO) cells, inhibition of hypusine biosynthesis followed by progressive arrest in cellular proliferation was observed with both N-mono- and N,N'-bisguanyl derivatives of 1,6-diaminohexane, 1,7-diaminoheptane, and 1,8-diaminooctane. Cells treated with these compounds showed no significant change in polyamine distribution, suggesting that the observed growth inhibition is not mediated through an interference with polyamine metabolism. N1-guanyl-1,7-diaminoheptane, the most potent inhibitor of **deoxyhypusine synthase** both in vitro and in cells, exhibited the highest antiproliferative activity toward CHO cells. No early cytotoxic effects were observed with this inhibitor, and its antiproliferative activity appeared to be reversible. Transport studies showed that N1-guanyl-1,7-diaminoheptane is actively taken up by the polyamine transport system. Mutant CHO cells defective in polyamine transport were found to be resistant to growth inhibition by this compound. The findings suggest that the antiproliferative effect of N1-guanyl-1,7-diaminoheptane is exerted intracellularly through inhibition of hypusine synthesis.

15/3,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07658292 93176104 PMID: 8439281
Effects of chronic 5'-([(Z)-4-amino-2-butenyl]methylamino)-5'-deoxy-adenosine (AbeAdo) treatment on polyamine and eIF-**5A** metabolism in AbeAdo-sensitive and -resistant L1210 murine leukaemia cells.
Byers T L; Wiest L; Wechter R S; Pegg A E
Department of Cellular and Molecular Physiology, M. S. Hershey Medical Center, Hershey, PA 17033.
Biochemical journal (ENGLAND) Feb 15 **1993**, 290 ( Pt 1) p115-21, ISSN 0264-6021 Journal Code: 2984726R
Contract/Grant No.: CA-18138; CA; NCI; CA-37606; CA; NCI; GM-26290; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have previously reported that prolonged chronic exposure to the S-adenosyl-L-methionine decarboxylase (AdoMetDC) inhibitor, 5'-([(Z)-4-amino-2-butenyl]methylamino)-5'-deoxy-adenosine (MDL 73811, AbeAdo), leads to cytostasis of L1210 cells [Byers, Ganem and Pegg (1992) Biochem. J. 287, 717-724]. Further studies to investigate the mechanism by which these effects are brought about were carried out by comparing an L1210-derived cell line (R20) that is resistant to AbeAdo with the parent cells. The R20 cells were derived by two rounds of AbeAdo-induced cytostasis followed by rescue with exogenous polyamines. Cytostasis was induced in L1210 cells treated for 12 days with 10 microM AbeAdo; however, exposure to up to 40 microM AbeAdo did not induce cytostasis in R20 cells. **Putrescine** levels were elevated and spermine levels were depleted in both treated L1210 and treated R20 cells. Spermidine was depleted in treated L1210 cells but was only partly reduced in treated R20 cells. AdoMetDC activity was below the limit of detection in treated L1210 cells but, although greatly reduced, could be measured in the treated R20 cells. The resistance of the R20 cells to the effects of AbeAdo on cell growth and spermidine depletion correlated with reduced AbeAdo accumulation by R20 cells. In the absence of spermidine synthesis, unhypusinated **eukaryotic** translation **initiation factor 5A** (eIF-**5A** ) accumulated in AbeAdo-treated L1210 cells. There was no detectable accumulation of unhypusinated eIF-**5A** in R20 cells. Unhypusinated eIF-**5A** accumulated during AbeAdo treatment was depleted in L1210 cells rescued by exogenous spermidine. These findings are consistent with the hypothesis that AbeAdo-induced cytostasis is due to the loss of hypusinated eIF-**5A** . However, spermine was able to rescue AbeAdo-treated L1210 cells without significantly reducing the unhypusinated eIF-**5A** accumulated during AbeAdo treatment, suggesting that only a small amount of the unmodified protein must be hypusinated to restore cell growth.

15/3,AB/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07548742 93075022 PMID: 1445235
Cytostasis induced in L1210 murine leukaemia cells by the S-adenosyl-L-methionine decarboxylase inhibitor 5'-([(Z)-4-amino-2-butenyl]methylamino)-5'-deoxyadenosine may be due to hypusine depletion.
Byers T L; Ganem B; Pegg A E
Department of Cellular and Molecular Physiology, M.S. Hershey Medical Center, Hershey, PA 17033.
Biochemical journal (ENGLAND) Nov 1 **1992**, 287 ( Pt 3) p717-24, ISSN 0264-6021 Journal Code: 2984726R
Contract/Grant No.: CA-18138; CA; NCI; CA-37606; CA; NCI; GM-26290; GM;

NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The effects of inhibition of the capacity to form spermidine and spermine on cell growth were investigated using murine leukaemia L1210 cells and 5'-([(Z)-4-amino-2-butenyl]methylamino)-5'-deoxyadenosine (MDL 73811, AbeAdo), an enzyme-activated irreversible inhibitor of S-adenosyl-L-methionine decarboxylase. **Putrescine** levels were increased 80-fold, and spermidine and spermine levels were greatly reduced after a 3-day exposure to a maximally inhibitory dose of 10 microM-AbeAdo. Addition of AbeAdo to the culture medium inhibited the growth of L1210 cells measured 3 days later in a dose-dependent manner, but, even at a dose of 10 microM, which was maximally effective, exposure to AbeAdo was not immediately cytostatic. However, the growth rate of L1210 cells chronically exposed to 10 microM-AbeAdo declined steadily until day 12, when the cells stopped growing. L1210 cells exposed to AbeAdo for 12 days could not be rescued from cytostasis by removal of the drug from the culture, but could be rescued by exposure to exogenous spermidine or spermine, indicating that the growth-inhibitory effects of AbeAdo were a result of spermidine and/or spermine depletion. It is suggested that elevated intracellular **putrescine** in AbeAdo-treated cells sustained limited growth in the absence of physiological levels of spermidine and spermine until certain critical and specific physiological role(s) fulfilled by spermidine (and/or spermine) became deficient resulting in cytostasis. N-(3-Aminopropyl)-1,4-diamino-cis-but-2-ene, a spermidine analogue that is a substrate for **deoxyhypusine synthase**, was able to mimic the effects of spermidine in reversing AbeAdo-induced cytostasis. Spermidine analogues such as 5,5-dimethylspermidine, which are not substrates for **deoxyhypusine synthase** , were not active in this way. These results provide evidence that the formation of hypusine in the protein-synthesis initiation factor eIF-5A may be a critical role of spermidine essential for cell growth.

15/3,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07280281 92210582 PMID: 1556119
Two isoforms of eIF-**5A** in chick embryo. Isolation, activity, and comparison of sequences of the hypusine-containing proteins.
Wolff E C; Kinzy T G; Merrick W C; Park M H
Laboratory of Cellular Development and Oncology, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20892.
Journal of biological chemistry (UNITED STATES) Mar 25 **1992**, 267 (9) p6107-13, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: AM-07319; AM; NIADDK; GM-26796; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
**Eukaryotic** translation **initiation factor 5A** (eIF-**5A** ) (older terminology, eIF-4D) is unique in that it contains the unusual amino acid hypusine (N epsilon-(4-amino-2-hydroxybutyl)lysine). Hypusine is formed by a post-translational event in which a specific lysine residue is modified by a structural contribution from spermidine. Metabolic labeling of chick embryo fibroblasts with [3H]spermidine or [3H]lysine gives rise to two distinct proteins, designated I (approximately 20 kDa and pI 5.6) and II (approximately 18 kDa and pI 5.35), that contain [3H]hypusine. Upon incubation with [3H]lysine the labeling of the two proteins followed a similar time course and showed approximately the same ratio over the 6-h incubation period. [3H]Hypusine-containing proteins from cells which had been cultured with [3H]spermidine were employed as tracers

for isolation of hypusine-containing proteins from whole chick embryos. Four such proteins were obtained. Two of these proteins, I and II, correspond to the two native proteins synthesized in chick embryo fibroblasts; the other two forms, Ia and IIa, displayed properties suggesting that they were derived from the native proteins, I and II, respectively, during purification. The amino acid compositions and the tryptic peptide maps of the 20-kDa protein (I) and the 18 kDa protein (II) suggest that they are closely related but distinct proteins. In fact, amino acid sequence analysis of the two major proteins revealed differences in the polypeptide backbone of the two proteins. In spite of structural differences, the two native forms (I and II), as well as the two altered forms (Ia and IIa), were effective in stimulating methionyl-puromycin synthesis, providing evidence that they are indeed functional isoforms of eIF-**5A**.

15/3,AB/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06910300 91217019 PMID: 1850732
Comparison of the activities of variant forms of eIF-4D. The requirement for hypusine or **deoxyhypusine**.
Park M H; Wolff E C; Smit-McBride Z; Hershey J W; Folk J E
Laboratory of Cellular Development and Oncology, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20892.
Journal of biological chemistry (UNITED STATES) May 5 **1991**, 266 (13) p7988-94, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: GM 22135; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
**Eukaryotic** protein synthesis **initiation factor** 4D (eIF-4D) (current nomenclature, eIF-**5A** ) contains the unique amino acid hypusine (N epsilon-(4-amino-2-hydroxybutyl)lysine). The first step in hypusine biosynthesis, i.e. the formation of the intermediate, **deoxyhypusine** (N epsilon-(4-aminobutyl)lysine), was carried out in vitro using spermidine, **deoxyhypusine synthase** , and ec-eIF-4D(Lys), an eIF-4D precursor prepared by over-expression of human eIF-4D cDNA in Escherichia coli. In a parallel reaction, using N-(3-aminopropyl)cadaverine in place of spermidine, a variant form of eIF-4D containing homodeoxyhypusine (N epsilon-(5-aminopentyl)lysine) was prepared. Evidence that N-(3-aminopropyl)cadaverine can also act as the amine substrate for **deoxyhypusine synthase** in intact cells was obtained by incubating **putrescine**- and spermidine-depleted Chinese hamster ovary cells with [3H]cadaverine. In these cells, in which [3H]cadaverine is readily converted to N-(3-aminopropyl) [3H]cadaverine, small amounts of [3H]homodeoxyhypusine and another 3H-labeled compound, presumed to be N epsilon-(5-amino-2-hydroxy[3H]pentyl)lysine, were found. eIF-4D stimulates methionyl-puromycin synthesis, an in vitro model assay for translation **initiation** . Whereas the unmodified precursor ec-eIF-4D(Lys) appeared inactive, the **deoxyhypusine**-containing form provided a significant degree of stimulation. The variant form containing homodeoxyhypusine, on the other hand, showed little or no activity. These findings emphasize the importance of hypusine or **deoxyhypusine** for the biological activity of eIF-4D and demonstrate the influence of both the length and chemical nature of its amino alkyl side chain.

15/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06342745 90036954 PMID: 2509461
The essential role of hypusine in **eukaryotic** translation

**initiation factor** 4D (eIF-4D). Purification of eIF-4D and its precursors and comparison of their activities.

Park M H
Laboratory of Cellular Development and Oncology, National Institute of Dental Research, Bethesda, Maryland 20892.

Journal of biological chemistry (UNITED STATES) Nov 5 **1989**, 264 (31) p18531-5, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

**Eukaryotic** translation **initiation factor** 4D (eIF-4D) is the only protein known to contain the amino acid, hypusine [N epsilon-(4-amino-2-hydroxybutyl)lysine]. This unusual amino acid is formed post-translationally by modification of a single specific lysine residue in an eIF-4D precursor protein. Two separate eIF-4D precursors, each of which contains a lysine residue in place of the hypusine residue and each of which thereby serves as a protein substrate for the hypusine modification, were purified from DL-2-difluoromethylornithine-treated Chinese hamster ovary cells by means of a five-step procedure. These two precursors termed PI and PII both have apparent molecular masses of approximately 17 kDa, indistinguishable from that of eIF-4D, but exhibit more acidic isoelectric points (5.1 and 5.25 for PI and PII, respectively, compared with 5.37 for eIF-4D). These physical characteristics, together with other properties, indicate that eIF-4D differs from PII only in possessing the hypusine residue in place of a lysine residue, whereas an additional structural difference exists between PI and eIF-4D. eIF-4D from CHO cells provides a significant enhancement of methionyl-puromycin synthesis, a model assay for translation **initiation** . Neither PI nor PII stimulates this in vitro system. These findings are the first direct evidence that hypusine is essential for the biological activity of eIF-4D.

15/3,AB/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05900947 88309816 PMID: 3136804

Isolation and characterization of an 18 kDa hypusine-containing protein from cultured NB-15 mouse neuroblastoma cells.

Chen K Y; Dou Q P
Department of Chemistry, Rutgers University, New Brunswick, NJ 08554.

Biochimica et biophysica acta (NETHERLANDS) Aug 19 **1988**, 971 (1) p21-8, ISSN 0006-3002 Journal Code: 0217513
Contract/Grant No.: AG03578; AG; NIA; CA24479; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

An 18 kDa protein can be metabolically labeled by [3H]**putrescine** or [3H]spermidine in various mammalian cells. The labeling is due to a post-translational modification of one lysine residue to hypusine using the aminobutyl moiety derived from spermidine. In view of the lack of knowledge of the function of this spermidine-modified protein, we decided to use the radioactivity associated with the [3H]spermidine-labeled 18 kDa protein as a tracer to develop a simple procedure for purifying this protein from cultured cells. We first screened more than 15 different affinity adsorbents for their ability to bind the labeled 18 kDa protein. This approach enabled us to develop a four-step procedure to purify the labeled 18 kDa protein from NB-15 mouse neuroblastoma cells. The procedure, including a Cibacron Blue column, an omega-aminooctyl-agarose, a Sepharose G-50, and a Mono Q column, resulted in an 800-fold purification of the labeled 18 kDa protein. Two-dimensional gel analysis of fractions enriched in the labeled 18 kDa protein revealed (i) the presence of isoforms of hypusine-containing 18 kDa protein, with pI values ranging from 4.7 to 5.2,

and (ii) the presence of an additional labeled protein with an apparent molecular mass of 22 kDa and a pI value of 5.0. The labeling intensity of the 22 kDa protein, however, was less than 5% of that of the 18 kDa protein. Peptide map analysis, using the V-8 proteinase digestion method, indicated that the 18 kDa hypusine-containing protein obtained from NB-15 cells was similar to **eukaryotic initiation factor** 4D isolated from rabbit reticulocytes.

15/3,AB/17 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)


13528838 BIOSIS NO.: 200200157659
Transcript level of the **eukaryotic initiation factor** **5A** gene peak at early G1 phase of the cell cycle in the dinoflagellate Crypthecodinium cohnii.
AUTHOR: Wong Joseph T Y(a); Chan Ka L(a); Lam Connie M C(a); Ghandhai Shanaz(a); David New(a)
AUTHOR ADDRESS: (a)Biology, Hong Kong University of Science and Technology, Clearwater Bay, Kowloon, Hong Kong**China
JOURNAL: Molecular Biology of the Cell 12 (Supplement):p278a Nov, **2001**
MEDIUM: print
CONFERENCE/MEETING: 41st Annual Meeting of the American Society for Cell Biology Washington DC, USA December 08-12, 2001
ISSN: 1059-1524
RECORD TYPE: Citation
LANGUAGE: English
2001

15/3,AB/18 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)


12853539 BIOSIS NO.: 200100060688
Cloning and expression of homospermidine **synthase** from Senecio vulgaris: A revision.
AUTHOR: Ober Dietrich; Harms Reiner; Hartmann Thomas(a)
AUTHOR ADDRESS: (a)Institut fuer Pharmazeutische Biologie der Technischen Universitaet Braunschweig, Mendelssohnstrasse 1, D-38106, Braunschweig: t.hartmann@tu-bs.de**Germany
JOURNAL: Phytochemistry (Oxford) 55 (4):p305-309 October, **2000**
MEDIUM: print
ISSN: 0031-9422
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Homospermidine **synthase**, which catalyses the first pathway-specific reaction in pyrrolizidine alkaloid biosynthesis, was cloned from root cultures of Senecio vulgaris and expressed in E. coli. The open reading frame encodes a protein of 370 amino acids with a molecular mass of 40,740 Da. The enzyme is strictly dependent on spermidine as aminobutyl donor since it cannot be substituted by **putrescine**. The homospermidine **synthase** from S. vulgaris showed 97.9 and 99.3% nucleic acid identity with two HSS sequences from the closely related species Senecio vernalis. This report also revises data from a previous publication (Kaiser, A., 1999. Cloning and expression of a cDNA encoding homospermidine **synthase** from Senecio vulgaris (Asteraceae) in Escherichia coli. Plant J. 19, 195-201.) that is incorrect.

**2000**

15/3,AB/19 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)


08823186 BIOSIS NO.: 199395112537
Effects of chronic 5'-((Z)-4-amino-2-butenyl)methylamino-5'-deoxyadenosine (AbeAdo) treatment on polyamine and eIF-**5A** metabolism in AbeAdo-sensitive and -resistant L1210 murine leukaemia cells.
AUTHOR: Byers Timothy L(a); Wiest Laurie; Wechter Rita S; Pegg Anthony E
AUTHOR ADDRESS: (a)Dep. Cellular Mol. Physiol., M.S. Hershey Med. Cent., Hershey, PA 17033**azakhstan
JOURNAL: Biochemical Journal 290 (1):p115-121 **1993**
ISSN: 0264-6021
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We have previously reported that prolonged chronic exposure to the S-adenosyl-L-methionine decarboxylase (AdoMetDC) inhibitor, 5'-(((Z)-4-amino-2-butenyl)methylamino)-5'-deoxyadenosine (MDL 73811, AbeAdo), leads to cytostasis of L1210 cells (Byers, Ganem and Pegg (1992) Biochem. J. 287, 717-724). Further studies to investigate the mechanism by which these effects are brought about were carried out by comparing an L1210-derived cell line (R20) that is resistant to AbeAdo with the parent cells. The R20 cells were derived by two rounds of AbeAdo-induced cytostasis followed by rescue with exogenous polyamines. Cytostasis was induced in L1210 cells treated for 12 days with 10 mu-M AbeAdo; however, exposure to up to 40 mu-M AbeAdo did not induce cytostasis in R20 cells. **Putrescine** levels were elevated and spermine levels were depleted in both treated L1210 and treated R20 cells. Spermidine was depleted in treated L1210 cells but was only partly reduced in treated R20 cells. AdoMetDC activity was below the limit of detection in treated L1210 cells but, although greatly reduced, could be measured in the treated R20 cells. The resistance of the R20 cells to the effects of AbeAdo on cell growth and spermidine depletion correlated with reduced AbeAdo accumulation by R20 cells. In the absence of spermidine synthesis, unhypusinated **eukaryotic** translation **initiation factor 5A** (**eIF5A**) accumulated in AbeAdo-treated L1210 cells. There was no detectable accumulation of unhypusinated eIF-**5A** in R20 cells. Unhypusinated eIF-**5A** accumulated during AbeAdo treatment was depleted in L1210 cells rescued by exogenous spermidine. These findings are consistent with the hypothesis that AbeAdo-induced cytostasis is due to the loss of hypusinated eIF-**5A**. However, spermine was able to rescue AbeAdo-treated L1210 cells without significantly reducing the unhypusinated eIF-**5A** accumulated during AbeAdo treatment, suggesting that only a small amount of the unmodified protein must be hypusinated to restore cell growth.

**1993**
? ds

| Set | Items | Description |
|---|---|---|
| S1 | 1088 | DHS OR (DEOXYHYPUSINE AND SYNTHASE) OR EIF5A OR ((EUKARYOTIC OR EUCARYOTIC) AND INITIATION AND FACTOR AND 5A) |
| S2 | 7 | S1 AND (ANTISENS? OR RIBOZYM?) |
| S3 | 4 | RD (unique items) |
| S4 | 17 | S1 AND APOPTOSIS? |
| S5 | 11 | RD (unique items) |
| S6 | 105 | S1 AND SPERMIDIN? |
| S7 | 65 | RD (unique items) |

S8 3 S7 AND APOPTOSIS?
S9 63 S7 AND PY<2002
S10 0 S1 AND DIAMO AND PROPANE
S11 0 S1 AND DIAMINO AND PROPANE
S12 0 S1 AND DIAMINO AND BUTANE
S13 33 S1 AND PUTRESCINE
S14 21 RD (unique items)
S15 19 S14 AND PY<2002
? s s1 and diamino and heptane
1088 S1
5375 DIAMINO
3158 HEPTANE
S16 2 S1 AND DIAMINO AND HEPTANE
? rd
...completed examining records
S17 1 RD (unique items)
? t s17/3,ab/all

17/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10993903 20558097 PMID: 11104695
Human **deoxyhypusine synthase** : interrelationship between binding of NAD and substrates.
Lee C H; Park M H
Building 30, Room 211, Oral and Pharyngeal Cancer Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD 20892-4340, USA.
Biochemical journal (ENGLAND) Dec 15 2000, 352 Pt 3 p851-7, ISSN 0264-6021 Journal Code: 2984726R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
**Deoxyhypusine synthase** catalyses the NAD-dependent transfer of the butylamine moiety from the polyamine, spermidine, to a specific lysine residue of a single cellular protein, **eukaryotic** translation-**initiation factor 5A** (**eIF5A**) precursor. The native enzyme exists as a tetramer of four identical subunits and contains four binding sites for NAD. The binding of spermidine and NAD was studied by a filtration assay. [(3)H]Spermidine binding to the enzyme was not detectable alone or in the presence of the **eIF5A** precursor, but was detected only in the presence of NAD or NADH, suggesting that a NAD/NADH-induced conformational change is required for the binding of spermidine. A strong NAD-dependent binding was also observed with a spermidine analogue, N(1)-guanyl-1, 7-**diamino**[(3)H]**heptane** (GC(7)), but not with [(14)C]putrescine or [(14)C]spermine. Although [(3)H]NAD binding to the enzyme occurred in the absence of spermidine, its affinity for the enzyme was markedly enhanced by spermidine, GC(7) and also by the **eIF5A** precursor. The maximum binding for NAD and spermidine was estimated to be approximately 4 molecules each/enzyme tetramer. The dependence of spermidine binding on NAD and the modulation of binding of NAD by spermidine and the **eIF5A** precursor suggest intricate relationships between the binding of cofactor and the substrates, and provide new insights into the reaction mechanism.
? s s1 and diamino and octane
1088 S1
5375 DIAMINO
3050 OCTANE
S18 0 S1 AND DIAMINO AND OCTANE
? s spermidin? and (octane or propane)
14336 SPERMIDIN?
3050 OCTANE
7556 PROPANE

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     S19     107  SPERMIDIN? AND (OCTANE OR PROPANE)
? s s19 and diamino?
             107  S19
           20294  DIAMINO?
     S20      65  S19 AND DIAMINO?
? s s20 and dhs
              65  S20
             809  DHS
     S21       0  S20 AND DHS
? s s20 and eif5a
              65  S20
              39  EIF5A
     S22       0  S20 AND EIF5A
? s s20 and 5a
              65  S20
           10337  5A
     S23       0  S20 AND 5A
? s s20 and deoxyhypusine
              65  S20
             189  DEOXYHYPUSINE
     S24       0  S20 AND DEOXYHYPUSINE
? s s20 and (eukaryotic or eucaryotic)
              65  S20
           48696  EUKARYOTIC
            3639  EUCARYOTIC
     S25       1  S20 AND (EUKARYOTIC OR EUCARYOTIC)
? t s25/3,ab/all

 25/3,AB/1     (Item 1 from file: 5)
DIALOG(R)File   5:Biosis Previews(R)
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03822788   BIOSIS NO.: 000075000861
WIDESPREAD OCCURRENCE OF NORSPERMIDINE AND NORSPERMINE IN EUKARYOTIC
  ALGAE
AUTHOR: HAMANA K; MATSUZAKI S
AUTHOR ADDRESS: COLL. MED. CARE AND TECHNOL., GUNMA UNIV., MAEBASHI, GUNMA
  371.
JOURNAL: J BIOCHEM (TOKYO) 91 (4). 1982. 1321-1328. 1982
FULL JOURNAL NAME: Journal of Biochemistry (Tokyo)
CODEN: JOBIA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Seven phyla of eukaryotic algae were analyzed to determine
  their contents of diamines and polyamines. The algae examined included
  Rhodophyta, Pyrrophyta, Chrysophyta, Phaeophyta, Euglenophyta,
  Chlorophyta and Charophyta. Putrescine and spermidine were detected
  in all the algae studied, while appreciable amounts of spermine were
  detected only in a few species of algae. 1,3-Diaminopropane,
  norspermidine and norspermine, which are chemical analogs of putrescine,
  spermidine and spermine, respectively, were widely distributed in
  various species of algae. There was no parallelism between the
  distribution patterns of putrescine derivatives and those of 1,3-
  diaminopropane derivatives. Cadaverine and agmatine were detected
  in multicellular marine algae. Homospermidine was detected sporadically
  in some algae. The biological and phylogenetical significance of
  polyamines in these lower eukaryotes is discussed.

1982
? ds

Set     Items   Description
S1       1088   DHS OR (DEOXYHYPUSINE AND SYNTHASE) OR EIF5A OR ((EUKARYOT-
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            IC OR EUCARYOTIC) AND INITIATION AND FACTOR AND 5A)
S2          7   S1 AND (ANTISENS? OR RIBOZYM?)
S3          4   RD (unique items)
S4         17   S1 AND APOPTOSIS?
S5         11   RD (unique items)
S6        105   S1 AND SPERMIDIN?
S7         65   RD (unique items)
S8          3   S7 AND APOPTOSIS?
S9         63   S7 AND PY<2002
S10         0   S1 AND DIAMO AND PROPANE
S11         0   S1 AND DIAMINO AND PROPANE
S12         0   S1 AND DIAMINO AND BUTANE
S13        33   S1 AND PUTRESCINE
S14        21   RD (unique items)
S15        19   S14 AND PY<2002
S16         2   S1 AND DIAMINO AND HEPTANE
S17         1   RD (unique items)
S18         0   S1 AND DIAMINO AND OCTANE
S19       107   SPERMIDIN? AND (OCTANE OR PROPANE)
S20        65   S19 AND DIAMINO?
S21         0   S20 AND DHS
S22         0   S20 AND EIF5A
S23         0   S20 AND 5A
S24         0   S20 AND DEOXYHYPUSINE
S25         1   S20 AND (EUKARYOTIC OR EUCARYOTIC)
? s s1 and diaminooctane
            1088  S1
              69  DIAMINOOCTANE
     S26       3  S1 AND DIAMINOOCTANE
? rd
...completed examining records
     S27       2  RD (unique items)
? t s27/3,ab/all

 27/3,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08292540   95050547   PMID: 7961711
    Antiproliferative effects of inhibitors of deoxyhypusine
synthase . Inhibition of growth of Chinese hamster ovary cells by
guanyl diamines.
  Park M H; Wolff E C; Lee Y B; Folk J E
  Enzyme Chemistry Section, NIDR, National Institutes of Health, Bethesda,
Maryland 20892.
  Journal of biological chemistry (UNITED STATES)  Nov 11 1994,  269  (45)
  p27827-32,  ISSN 0021-9258  Journal Code: 2985121R
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  Certain guanyl diamines are effective inhibitors of deoxyhypusine
synthase (Jakus, J., Wolff, E. C., Park, M. H., and Folk, J. E.
(1993) J. Biol. Chem. 268, 13151-13159), the first enzyme involved in the
biosynthesis of the unusual amino acid hypusine (N
epsilon-(4-amino-2-hydroxybutyl)lysine). Evidence that hypusine is
implicated in cell growth prompted this study of the cellular effects of
these inhibitors. In Chinese hamster ovary (CHO) cells, inhibition of
hypusine biosynthesis followed by progressive arrest in cellular
proliferation was observed with both N-mono- and N,N'-bisguanyl derivatives
of 1,6-diaminohexane, 1,7-diaminoheptane, and 1,8-diaminooctane .
Cells treated with these compounds showed no significant change in
polyamine distribution, suggesting that the observed growth inhibition is
not mediated through an interference with polyamine metabolism.
N1-guanyl-1,7-diaminoheptane, the most potent inhibitor of
```

**deoxyhypusine synthase** both in vitro and in cells, exhibited the highest antiproliferative activity toward CHO cells. No early cytotoxic effects were observed with this inhibitor, and its antiproliferative activity appeared to be reversible. Transport studies showed that N1-guanyl-1,7-diaminoheptane is actively taken up by the polyamine transport system. Mutant CHO cells defective in polyamine transport were found to be resistant to growth inhibition by this compound. The findings suggest that the antiproliferative effect of N1-guanyl-1,7-diaminoheptane is exerted intracellularly through inhibition of hypusine synthesis.

27/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05928204 89008419 PMID: 3139668
Cell-free synthesis of deoxyhypusine. Separation of protein substrate and enzyme and identification of 1,3-diaminopropane as a product of spermidine cleavage.
Park M H; Wolff E C
Laboratory of Cellular Development and Oncology, National Institute of Dental Research, Bethesda, Maryland 20892.
Journal of biological chemistry (UNITED STATES) Oct 25 1988, 263 (30) p15264-9, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The post-translational formation of hypusine (N epsilon-(4-amino-2-hydroxybutyl)lysine) occurs in a precursor of **eukaryotic initiation factor** 4D by way of two major steps: 1) transfer of the 4-aminobutyl moiety from spermidine to the epsilon-amino group of a specific lysine residue to form an intermediate, deoxyhypusine; 2) hydroxylation of the deoxyhypusine residue to form hypusine. The initial step of this modification, deoxyhypusine synthesis, was studied in fractionated lysates of Chinese hamster ovary cells, untreated, or treated with alpha-difluoromethylornithine (DFMO); the enzyme(s) and the protein substrate (**eukaryotic initiation factor** 4D precursor) were separated. The enzyme activity was found in the 0-45% ammonium sulfate fraction from both untreated and DFMO-treated cells. The protein substrate was detected in the 45-75% ammonium sulfate fraction from cells depleted of spermidine by treatment with DFMO, but not in any fraction from untreated cells. Upon further purification of the protein substrate by ion exchange chromatography, the requirement for a pyridine nucleotide, notably NAD+, became apparent. Free 1,3-diaminopropane was identified as a spermidine cleavage product formed concurrently with the 4-aminobutyl transfer step of deoxyhypusine synthesis. Compounds structurally related to spermidine, e.g. caldine, N4-benzylspermidine, homospermidine, and a spermine homologue, thermine, as well as 1,7-diaminoheptane, 1,8-**diaminooctane** , and 1,9-diaminononane caused significant inhibition of deoxyhypusine synthesis presumably due to competition with spermidine. 1,3-Diaminopropane exhibited a potent inhibition of deoxyhypusine formation, probably through a different mechanism.
? ds

| Set | Items | Description |
|---|---|---|
| S1 | 1088 | DHS OR (DEOXYHYPUSINE AND SYNTHASE) OR EIF5A OR ((EUKARYOTIC OR EUCARYOTIC) AND INITIATION AND FACTOR AND 5A) |
| S2 | 7 | S1 AND (ANTISENS? OR RIBOZYM?) |
| S3 | 4 | RD (unique items) |
| S4 | 17 | S1 AND APOPTOSIS? |
| S5 | 11 | RD (unique items) |
| S6 | 105 | S1 AND SPERMIDIN? |
| S7 | 65 | RD (unique items) |
| S8 | 3 | S7 AND APOPTOSIS? |

```
S9          63   S7 AND PY<2002
S10          0   S1 AND DIAMO AND PROPANE
S11          0   S1 AND DIAMINO AND PROPANE
S12          0   S1 AND DIAMINO AND BUTANE
S13         33   S1 AND PUTRESCINE
S14         21   RD (unique items)
S15         19   S14 AND PY<2002
S16          2   S1 AND DIAMINO AND HEPTANE
S17          1   RD (unique items)
S18          0   S1 AND DIAMINO AND OCTANE
S19        107   SPERMIDIN? AND (OCTANE OR PROPANE)
S20         65   S19 AND DIAMINO?
S21          0   S20 AND DHS
S22          0   S20 AND EIF5A
S23          0   S20 AND 5A
S24          0   S20 AND DEOXYHYPUSINE
S25          1   S20 AND (EUKARYOTIC OR EUCARYOTIC)
S26          3   S1 AND DIAMINOOCTANE
S27          2   RD (unique items)
? s s1 and diaminopropane
            1088  S1
             840  DIAMINOPROPANE
     S28       9  S1 AND DIAMINOPROPANE
? rd
...completed examining records
     S29       5  RD (unique items)
? t s29/3,ab/all

 29/3,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10408253   99407273   PMID: 10476066
   Cloning and expression of a cDNA encoding homospermidine synthase
 from Senecio vulgaris (Asteraceae) in Escherichia coli.
  Kaiser A
  Institute of Pharmaceutical Biology, Braunschweig, Germany.
akaiser@parasit.meb.uni-bonn.de
  Plant journal : for cell and molecular biology (ENGLAND) Jul 1999, 19
 (2) p195-201, ISSN 0960-7412 Journal Code: 9207397
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  The enzyme homospermidine synthase catalyzes the NAD+-dependent
conversion of 2 mol putrescine into homospermidine. Instead of putrescine,
spermidine can substitute for the first putrescine moiety in plants, in
which case diaminopropane instead of ammonia is released. The enzyme
facilitates the formation of the 'uncommon' polyamine homospermidine which
is an important precursor in the biosynthesis of pyrrolizidine alkaloids.
The first plant homospermidine synthase was purified to apparent
chemical homogenity from the root tissue culture Senecio vernalis
(Asteraceae) (Bottcher et al. 1994, Can. J. Chem. 72, 80-85; Ober 1997,
Dissertation). Four endopeptidase LysC fragments were sequenced from the
purified protein. With the aid of degenerate primers against these
peptides, a cDNA encoding homospermidine synthase was now cloned and
characterized from Senecio vulgaris. The nucleotide sequence of the cloned
cDNA revealed an open reading frame of 1155-base pairs containing 385 amino
acids with a predicted Mr of 44500. GenBank research revealed that the
deduced amino acid sequence shows 59% identity to human deoxyhypusine
 synthase. The homospermidine synthase encoding cDNA was
subcloned into the expression vector pet15b and overexpressed in E. coli.
The recombinant enzyme formed upon expression catalyzed homospermidine
synthesis.
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29/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09441028 97332674 PMID: 9188485
Enzyme-substrate intermediate formation at lysine 329 of human **deoxyhypusine synthase**.
Wolff E C; Folk J E; Park M H
Oral and Pharyngeal Cancer Branch, NIDR, National Institutes of Health, Bethesda, Maryland 20892-4340, USA.
Journal of biological chemistry (UNITED STATES) Jun 20 1997, 272 (25) p15865-71, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
**Deoxyhypusine** (Nepsilon-(4-aminobutyl)lysine) is the key intermediate in the posttranslational synthesis of the unique amino acid, hypusine (Nepsilon-(4-amino-2-hydroxybutyl)lysine). **Deoxyhypusine synthase** catalyzes the formation of **deoxyhypusine** by conjugation of the butylamine moiety of spermidine to the epsilon-amino group of one specific lysine residue of the **eukaryotic** translation **initiation factor 5A** (eIF-**5A**) precursor protein. However, in the absence of the eIF-**5A** precursor, catalysis involves only the NAD-dependent cleavage of spermidine to generate 1,3-**diaminopropane** and a putative 4-carbon amine intermediate that gives rise to Delta1-pyrroline. We have obtained evidence for a covalent enzyme-substrate intermediate that accumulates in the absence of the eIF-**5A** precursor. Incubation of human recombinant enzyme with [1, 8-3H]spermidine and NAD, followed by reduction with NaBH3CN, resulted in specific radiolabeling of the enzyme. The radioactive component in the reduced enzyme intermediate was identified as **deoxyhypusine** and was shown to occur at a single locus. The fact that labeled **deoxyhypusine** was found after treatment with a reducing agent suggests an intermediate with the butylamine moiety derived from spermidine attached through an imine linkage to the epsilon-amino group of a specific lysine residue of the enzyme. This residue has been identified as lysine 329. Separate experiments showing efficient transfer of labeled butylamine moiety from enzyme intermediate to eIF-**5A** precursor strongly support a reaction mechanism involving an imine intermediate.

29/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09423703 97306062 PMID: 9163530
Biochemical and immunological characterization of **deoxyhypusine synthase** purified from the yeast Saccharomyces carlsbergensis.
Abid M R; Sasaki K; Titani K; Miyazaki M
Department of Molecular Biology, School of Science, Nagoya University, Chikusa-ku.
Journal of biochemistry (JAPAN) Apr 1997, 121 (4) p769-78, ISSN 0021-924X Journal Code: 0376600
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
**Deoxyhypusine synthase** catalyzes the NAD+-dependent formation of **deoxyhypusine** in the eIF-5A precursor protein by transferring the 4-aminobutyl moiety of spermidine. This enzyme has recently been shown to be essential for cell viability and growth of yeast [Sasaki, K., Abid, M.R., and Miyazaki, M. (1996) FEBS Lett. 384, 151 154]. We have purified and characterized the enzyme from the yeast Saccharomyces carlsbergensis. The yeast and recombinant enzymes had a specific activity of 1.21 to 1.26

pmol per min per pmol of protein, and recognized both the eIF-5A precursor proteins almost equally as judged from their similar K(m) and V(max) values. Size exclusion chromatography and SDS-PAGE indicated that the active form of the enzyme is a homotetramer consisting of 43-kDa subunits. The enzyme showed a strict specificity for its substrates, NAD+, spermidine and eIF-5A precursor protein. Among all the substrates tested, only NAD+ showed a protective effect against heat inactivation of the enzyme suggesting that NAD+ initiates some conformational change in the enzyme. NADH exhibited a strong non-competitive inhibition (product inhibition). Unexpectedly, FAD, FMN, and riboflavin showed a moderate competitive inhibition. The competitive inhibition by diamines was maximal with compounds resembling spermidine in carbon chain length. 1,3-**Diaminopropane** inhibited the enzyme strongly in a competitive manner (product inhibition). On the other hand, putrescine did not inhibit the enzyme or act as a substrate. A polyclonal antibody raised against the yeast recombinant enzyme specifically inhibited **deoxyhypusine synthase** activity. The cross-reactivity (by Western blotting) of this antibody with the crude extracts varied depending on the source, indicating species specificity.

29/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06498533 90202944 PMID: 2108161
Cleavage of spermidine as the first step in **deoxyhypusine** synthesis. The role of NAD.
Wolff E C; Park M H; Folk J E
Laboratory of Cellular Development and Oncology, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20892.
Journal of biological chemistry (UNITED STATES) Mar 25 1990, 265 (9) p4793-9, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The biosynthesis of **deoxyhypusine** (N-(4-aminobutyl)lysine) occurs by the transfer of the 4-aminobutyl moiety of spermidine to a specific lysine residue in a precursor of **eukaryotic** translation **initiation factor** 4D (eIF-4D). **Deoxyhypusine synthase**, the enzyme that catalyzes this reaction, was purified approximately 700-fold from rat testis. The Km values for the substrates, spermidine, the eIF-4-D precursor protein, and NAD+, were estimated as approximately 1, 0.08, and 30 microM, respectively. After incubation of partially purified enzyme with [1,8-3H]spermidine, NAD+, and the eIF-4D precursor, equal amounts of radioactivity were found in free 1,3-**diaminopropane** and in protein-bound **deoxyhypusine**. However, when the protein substrate (eIF-4D precursor) was omitted, radioactivity was found in 1,3-**diaminopropane** and in delta 1-pyrroline in nearly equal quantities, providing evidence that the cleavage of spermidine occurs, albeit at a slower rate, in the absence of the eIF-4D precursor. That NAD+, which is required for this reaction, functions as the hydrogen acceptor was demonstrated by the fact that radioactivity from spermidine labeled with 3H at position 5 is found in NADH as well as in delta 1-pyrroline. Transfer of this hydrogen from spermidine to the re face of the nicotinamide ring of NAD+, as determined by the use of dehydrogenases of known stereospecificity, defines the first step of **deoxyhypusine** synthesis as a pro-R, or A, stereospecific dehydrogenation. Based on these findings, an enzyme mechanism involving imine intermediate formation is proposed.

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Cell-free synthesis of deoxyhypusine. Separation of protein substrate and enzyme and identification of 1,3-**diaminopropane** as a product of spermidine cleavage.

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The post-translational formation of hypusine (N epsilon-(4-amino-2-hydroxybutyl)lysine) occurs in a precursor of **eukaryotic initiation factor** 4D by way of two major steps: 1) transfer of the 4-aminobutyl moiety from spermidine to the epsilon-amino group of a specific lysine residue to form an intermediate, deoxyhypusine; 2) hydroxylation of the deoxyhypusine residue to form hypusine. The initial step of this modification, deoxyhypusine synthesis, was studied in fractionated lysates of Chinese hamster ovary cells, untreated, or treated with alpha-difluoromethylornithine (DFMO); the enzyme(s) and the protein substrate (**eukaryotic initiation factor** 4D precursor) were separated. The enzyme activity was found in the 0-45% ammonium sulfate fraction from both untreated and DFMO-treated cells. The protein substrate was detected in the 45-75% ammonium sulfate fraction from cells depleted of spermidine by treatment with DFMO, but not in any fraction from untreated cells. Upon further purification of the protein substrate by ion exchange chromatography, the requirement for a pyridine nucleotide, notably NAD+, became apparent. Free 1,3-**diaminopropane** was identified as a spermidine cleavage product formed concurrently with the 4-aminobutyl transfer step of deoxyhypusine synthesis. Compounds structurally related to spermidine, e.g. caldine, N4-benzylspermidine, homospermidine, and a spermine homologue, thermine, as well as 1,7-diaminoheptane, 1,8-diaminooctane, and 1,9-diaminononane caused significant inhibition of deoxyhypusine synthesis presumably due to competition with spermidine. 1,3-**Diaminopropane** exhibited a potent inhibition of deoxyhypusine formation, probably through a different mechanism.